# EVALUATION OF THE CHEMOPROTECTIVE ACTIVITY OF 70% METHANOLIC EXTRACT OF GMELINA ARBOREA STEM BARK AGAINST CYCLOPHOSPHAMIDE INDUCED TOXICITY

**Thesis Submitted to**

***The Tamilnadu Dr.M.G.R Medical University, Chennai***

***In partial fulfillment of the requirements***

***for the award of the Degree of***

***M*ASTER OF PHARMACY**

**IN**

**PHARMACOLOGY**


Submitted by

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## Under the guidance of


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**RVS COLLEGE OF PHARMACEUTICAL SCIENCES, SULUR, COIMBATORE**

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# CERTIFICATE

This is to certify that this project work entitled "**EVALUATION OF THE CHEMOPROTECTIVE ACTIVITY OF 70% METHANOLIC EXTRACT OF GMELINA ARBOREA STEM BARK AGAINST CYCLOPHOSPHAMIDE INDUCED TOXICITY"** submitted in partial fulfillment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmacology** to **The Tamilnadu Dr. M.G.R Medical University, Chennai** is a bonafied work carried out by **Mr. AMARRAJ VAZHOOR (Reg. No: 26116392)** at **Amala Cancer Research Centre, Thrissur, Kerala,** and completed the work at **Department of Pharmacology, R.V.S College of Pharmaceutical Sciences**, **Sulur, Coimbatore-641402** under the supervision and guidance of **Dr. D. Benito Johnson** (Institutional Guide) and **Dr. Jose Padikkala** (Industrial Guide) during the academic year 2012-2013.

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# EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **" EVALUATION OF THE CHEMOPROTECTIVE ACTIVITY OF 70% METHANOLIC EXTRACT OF *GMELINA ARBOREA* STEM BARK AGAINST CYCLOPHOSPHAMIDE INDUCED TOXICITY "** submitted by **Mr. AMARRAJ VAZHOOR (Reg.No: 26116392)** to **THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY, Chennai** in partial fulfillment for the Degree of **Master of pharmacy** in **Pharmacology** is a bonafied work carried out during the academic year by the candidate at the Department of Pharmacology, R.V.S College of Pharmaceutical Sciences, Sulur, Coimbatore and was evaluated by us.

**Examination centre:**

**Date:**

**Internal Examiner** **External Examiner**

# DECLARATION

I **Amarraj Vazhoor**, hereby declare that the report of the project work on **"Evaluation of the chemoprotective activity of 70% methanolic extract of *Gmelina arborea* stem bark against cyclophosphamide induced toxicity"** submitted to the **The Tamilnadu Dr. MGR Medical University** in partial fulfillment of the requirement for the award of **Master of Pharmacy** in **Pharmacology** is a bonafide record of the work done by me under the supervision and guidance of **Dr.Jose Padikkala**, Professor, Amala Cancer Research Centre, Thrissur and under the internal supervision of **Dr.D.Benito Johnson,** Professor, Head of Department of Pharmacology, R.V.S College of Pharmaceutical Sciences, Sulur, Coimbatore during the academic year 2012-2013 and it has not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or other similar title to any candidate of any University.

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**(AMARRAJ VAZHOOR)**

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| % | : | Percent sign |
| µl | : | micro litre |
| $^{0}$C | : | Degree celsius |
| ALP | : | Alkaline phosphatise |
| ALT | : | Alanine Aminotransferase |
| ANOVA | : | Analysis of variation |
| AST | : | Aspartate aminotransferase |
| cAMP | : | Cyclic adenosine monophosphate |
| cells/mm$^{3}$ | : | Cells per cubic millimetre |
| Cm | : | Centimetre |
| CML | : | Chronic myelocytic leukemia |
| CPK | : | Creatine phosphokinase |
| CTX | : | Cyclophosphamide |
| DNA | : | Deoxyribonucleic acid |
| DOX | : | Doxorubicin |
| FDA | : | Food and drug administration |
| Fig | : | Figure |
| FSH | : | Follicle-stimulating hormone |
| g/dl | : | grams per decilitre |
| GA | : | *Gmelina arborea* |
| GAHC | : | *Gmelina arborea* high concentration |
| GALC | : | *Gmelina arborea* low concentration |
| gm | : | Gram |
| GPx | : | Glutathione peroxidase |
| GSH | : | Glutathione |
| Hr | : | Hour |
| i.p | : | Intraperitonially |
| IU/L | : | International units per litre |
| KA | : | King-Armstrong |
| LDH | : | Lactate dehydrogenase |
| LH | : | Luteinizing hormone |

| | | |
|---|---|---|
| LPO | : | Lipid peroxidation |
| M | : | molar |
| MAP | : | Mitogen-activated protein |
| mg/dl | : | milligrams per decilitre |
| mg/kg b.wt | : | milligram per kilogram body weight |
| mg/kg | : | 1 milligram per kilogram |
| $mg/m^2$ | : | 1 milligram per square meter |
| Min | : | minute |
| Ml | : | Milli liter |
| Mm | : | Millimeter |
| mM | : | millimolar |
| $mm^3$ | : | Cubic millimetre |
| MTX | : | Methotrexate |
| nm | : | nanometer |
| OD | : | Optical density |
| RNA | : | Ribonucleic acid |
| ROS | : | Reactive oxygen species |
| SD | : | Standard deviation |
| SIADH | : | Syndrome of inappropriate antidiuretic hormone secretion |
| SOD | : | Superoxide dismutase |
| WBC | : | White blood cells |

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# 1. INTRODUCTION

Cancer is a group of more than 100 different diseases which is characterized by uncontrolled cellular growth, distant metastases and local tissue invasion (Kaufman *et al*., 1996). Carcinogenesis is a multi-step process that includes initiation phase, promotion phase, conversion phase and finally progression phase. The growth of both normal and cancerous cells is genetically controlled by the balance or imbalance of oncogene, protooncogene and tumor suppressor gene. Multiple genetic mutations are required to convert normal cells to cancerous cells. Apoptosis and cellular senescence (aging) are normal mechanisms for cell death. Cancer cells are genetically unstable, which results in tumor masses of heterogeneous cells and makes the cancer a "moving target" for drug therapy. Existence of many different clones of cancer cells in most patients provides the rationale for use of cancer drugs in combination and is the likely reason for failure of cancer drug therapy to cure most patients with advanced cancer.

The goals of cancer treatment include cure, prolongation of life and relief of symptoms. Surgery and radiation therapy provide the best chance of cure for patients with localized cancers, but systemic treatment methods are required for systemic cancers. Long-term complications of cancer treatment, such as infertility, secondary malignancies, effects on physical or intellectual development and major organ damage, can negatively affect health and quality of life for cancer survivors.

Traditionally, cancer drugs were discovered through large-scale testing of synthetic chemicals and natural products against rapidly proliferating animal tumor systems, primarily murine leukemias (Chabner and Roberts, 2005). Most of the agents that are discovered in the first two decades of cancer chemotherapy (1950 to 1970) interacted with DNA or its precursors, thereby inhibiting the synthesis of new genetic material or causing irreparable damage to DNA. In recent years, the discovery of new agents has extended from the more conventional natural products such as paclitaxel and semisynthetic agents such as etoposide which targets the proliferative process. Initial success in characterizing unique tumor antigens and oncogenes has introduced new therapeutic opportunities in therapy. The bcr-abl translocation in chronic myelocytic leukemia (CML) encodes a tyrosine kinase which is essential to cell proliferation and survival. Inhibition of the kinase by imatinib (STI-571), a new molecularly targeted drug which has become the standard treatment for CML results in hematologic and cytogenic remission in the majority of patients. Similarly the targeted immunological approaches

use monoclonal antibodies against tumor-associated antigens such as the her-2/neu receptor in cancer cells (breast), which is in conjunction with cytotoxic drugs (Slamon *et al.,* 2001).

Human beings have relied on natural products as a resource of drugs for past thousands of years. Plant-based drugs that have formed the basis of traditional medicine systems that have been used for centuries in many countries such as Egypt, China and India (Balandrin *et al.,* 1993). Today plant-based drugs continue to play an essential role in human health care. It has been estimated by the World Health Organization that, about 80% of the population of the world rely mainly on traditional medicines for their primary health care (Farnsworth *et al.,* 1985). Natural products also play an important role in the health care of the remaining 20% people around the world, who mainly reside in developed countries. At present, at least 119 chemicals which are derived from 90 plant species, can also be considered as important drugs in one or more countries (Arvigo R *et al.,* 1993).

Plant secondary metabolites have proved to be an excellent reservoir of new medical compounds. Many anti-cancer agents have been isolated from various plant sources like *Catharanthus roseus*, *Taxus brevifolia*, *Podophyllum* species, *Camptotheca acuminate*, *Betula alba*, *Cephalotaxus* species, *Curcuma longa*, *Erythroxylum pervillei*, *Centaurea schischkinii, Ipomoeca batatas* and many others. Still, scientists are attempting to explore the availability of anti-cancerous compounds in unexplored plant species.

**Carcinogenesis**

The mechanism by which cancers occur is incompletely understood. A cancer or neoplasm, is thought to develop from a cell in which the normal mechanisms for control of growth and proliferation are altered. Current evidence supports the concept of carcinogenesis as a multistage process that is genetically regulated. Initiation is the first step in this process, which requires exposure of normal cells to carcinogenic substances. These carcinogens certainly produce genetic damage, if not repaired that results in irreversible cellular mutations. This mutated cell has an altered response to its environment and a selective growth advantage, providing the potential to develop into a clonal population of neoplastic cells. During the second phase known as promotion, carcinogens or other factors that alter the environment to favour the growth of the mutated cell population over normal cells. The primary difference between initiation and

promotion is that promotion is a reversible process. In fact, because it is reversible, the promotion phase may be the target of future chemoprevention strategies including changes in lifestyle and diet. At some point, however, the mutated cell becomes cancerous (conversion or transformation). Depending on the type of cancer, 5 to 20 years may elapse between the carcinogenic phases and the development of a clinically detectable cancer. The final stage of neoplastic growth called as progression, which involves further genetic changes leading to increased proliferation of the cells. The major elements of this phase include tumor invasion into local tissues and the development of metastases (Andrea Iannucci *et al.*, 2005).

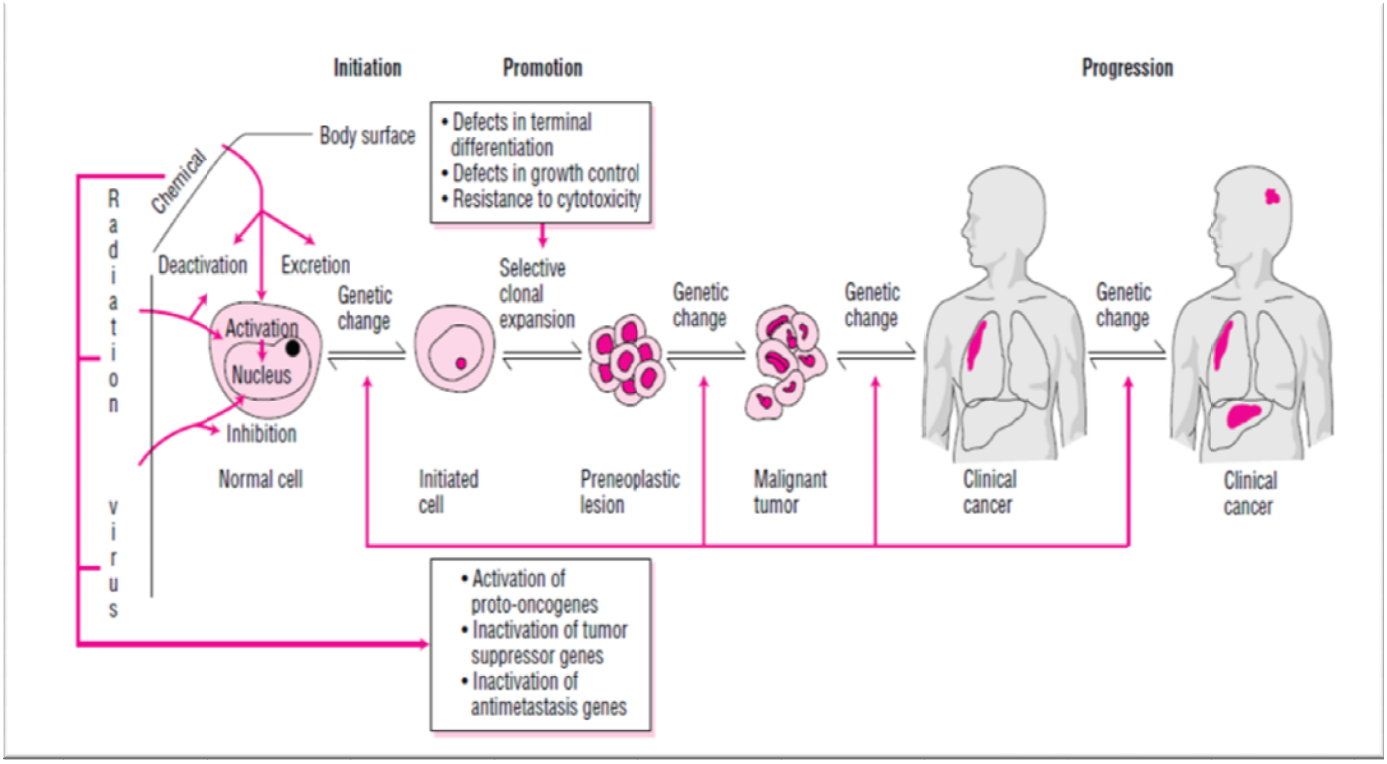



**Fig: 1** - Multistage model of carcinogenesis (Weston *et al.*,2003)

**Genetic basis of cancer**

Cancer has been described as "a malady of genes that arising from genetic damage of diverse sorts and are leading to distortions of either expression or biochemical function of genes" (Dupont W D and Page D L.,1991).

A normal cell turns into a cancer cell because of one or more mutations in its DNA, which can be acquired or inherited. However, carcinogenesis is a complex multistage process which usually involves more than one genetic change as well as other epigenetic factors such as hormonal, carcinogens and tumour promoter effects etc. that do not themselves results in cancer but which can increase the chances that the genetic mutation(s) will eventually result in cancer.

There are two main categories of genetic change that are important.

- The activation of proto-oncogenes to oncogenes. The Proto-oncogenes are genes that normally control cell division, differentiation and apoptosis, but can be converted to oncogenes.

- The inactivation of tumour suppressor genes. The Normal cells contain genes which posses the ability to suppress malignant change-termed tumour suppressor genes (antioncogenes) and there is now good evidence that mutations of these genes results in many different type of cancers. The loss or lack of function of tumour suppressor genes can be an important event in carcinogenesis.

**THE SPECIAL CHARACTERISSTICS OF CANCER CELLS**

1. UNCONTROLLED PROLIFERATION

   Transformation of proto-oncogenes into oncogenes or inactivation of tumour suppressor genes can alter autonomy of growth on a cell and thus result in uncontrolled proliferation by producing changes in several cellular systems including:

- Growth factors, signalling pathways and their receptors.
- The cell cycle transducers, for example the cdk inhibitors or cyclin-dependent kinases (cdks), cyclins.
- The apoptotic machinery that normally remove abnormal cells.
- Telomerase expression.
- Local blood vessels which are resulting from tumour-directed angiogenesis.

2. DEDIFFERENTIATION AND LOSS OF FUNCTION

The multiplication of normal cells in a tissue begins with division of the undifferentiated stem cells giving rise to its daughter cells. These daughter cells differentiate to become the mature cells of the relevant tissue that are ready to perform their functions. For example, when a fibroblasts mature, they can able to secrete and organise extracellular matrix and mature muscle cells are capable of contraction. The main characteristics of cancer cells is that they can dedifferentiate to varying degrees.

3. INVASIVENESS

Normal cells are not found outside their 'designated' tissue of origin; for example, liver cells are not found in the bladder and pancreatic cells are not found in the testis. This is because, during differentiation and tissue or organ growth the normal cells develop certain spatial relationships with respect to each other and these are maintained by various tissue-specific survival factors that prevent apoptosis. In this way, any cells that escape accidentally lose these survival signals and die. Consequently, although the cells of the normal mucosal epithelium of the rectum proliferate continuously as the lining is shed, they remain as a lining of epithelium. A cancer of the rectal mucosa invades other tissues forming the rectum and may even invade the tissues of other organs in pelvic. Cancer cells not only lost, through mutation, the restraints can act on normal cells, but they also secrete enzymes (e.g. metalloproteinases) that break down the extracellular matrix that enables them to move around.

4. METASTASES

Metastases are secondary tumours formed by cells that have been released from the initial or primary tumour and have reached other sites through blood vessels or lymphatics, or as a result of being shed into the body cavities. The metastases are the principal cause of mortality and morbidity in most cancers and cause a major problem for cancer therapy (Rang *et al.,* 2007).

**CANCER TREATMENT MODALITIES**

Choice of cancer treatment is influenced by several other factors, including the specific characteristics of the cancer, the overall condition and whether the goal of treatment is to cure cancer and keep the cancer from spreading or to relieve the symptoms

that are caused by cancer. One or more treatment modalities may be used to provide you with the most effective treatments. And it is common to use several treatment modalities together (concurrently) or in sequence with the goal of preventing reocurrence. This can referred to as multi-modality treatment of the cancer. Depending on these factors, you may receive one or more of the following

- Surgery

Surgery is used to diagnose, determine its stage and to treat cancer. One of the common type of surgery that may be used to help with diagnosing cancer is a biopsy. When surgery is used for treatment of cancer and tissue that are adjacent to the cancer are typically removed.

- Chemotherapy

Chemotherapy is any treatment involving the use of drugs to kill cancer cells. Cancer chemotherapy consist of single drugs or combinations of drugs and that can be administered through a vein, delivered orally in the form of a pill or injected into a body cavity. Chemotherapy is different from radiation therapy or surgery, the cancer-fighting drugs circulate in the blood to different parts of the body where the cancer may have spread and can kill or eliminate cancers cells at sites great distances from the original cancer site. Hence chemotherapy is considered a systemic treatment.

- Radiation therapy

Radiation therapy or radiotherapy uses high-energy rays to kill or damage cancer cells by preventing them from dividing and growing. Radiation therapy is an local treatment that are used to eliminate or eradicate visible tumors similar to surgery,. Radiation may be used to control or cure cancer or to relieve some of the symptoms caused by cancer.

- Hormonal therapy

Hormones are naturally occurring substances in the body that can stimulate the growth of hormone sensitive tissues such as the prostate gland or breast. When cancer arises in prostate or breast tissue, its growth and spread can also be caused by the body's own hormones. Hence the drugs that blocks hormone production or change the way in

which hormones work or removal of organs that secrete hormones such as the testicles or ovaries are ways of fighting cancer.

- Targeted therapy

  A targeted therapy is one that is designed to treat only the cancer cells and minimize damage to normal, healthy cells. Cancer treatments that "target" cancer cells may offer the advantage of reduced treatment-related side effects and improved outcomes.

- Biological therapy

Biological therapy is referred to by many terms that includes immunologic therapy, biotherapy or immunotherapy. Biological therapy is a type of treatment in which that uses the body's own immune system to facilitate the killing of cancer cells. Types of biological therapy include interleukin, interferon, colony stimulating factors (cytokines), monoclonal antibodies and vaccines.

- Personalized Cancer Care

There is no longer a "one-size-fits-all" approach in cancer treatment. Among patients with the same type of cancer, the behavior of the cancer may vary and its response to treatment can vary. By knowing the reasons for this variation, researchers have begun to realize the need for more personalized cancer treatment.

## CHEMOTHERAPY

Paul Ehrlich introduced the term chemotherapy in 1907 to describe his important early studies of the tsetse fly–borne parasite, *Trypanosoma brucei* that causes African trypanosomiasis (sleeping sickness). The term chemotherapy initially referring to antiparasitic therapy now called more broadly to the use of any chemical compound that selectively acts on microbes, viral or cancer. Ehrlich had previously developed selective chemical stains for the microscopic examination of *Mycobacterium tuberculosis* and other microorganisms, using the coal-tar derivative dyes. He tested many of these organic compounds for their selective toxicity against trypanosomes but failed to find an effective nontoxic antischistosomal agent. Turning to the chemotherapy of syphilis, Ehrlich eventually discovered the arsenical compound salvarsan, which was both remarkably

nontoxic to humans and remarkably toxic against a number of treponemal diseases including syphilis and yaws. Ehrlich called salvarsan the magic bullet.

Humans were not the first to exploit the selective toxicity of chemicals. Many fungi and bacteria make toxic substances that kill or suppress the growth of competing microorganisms or facilitate infection of a host. Plants make a vast array of toxins for their self-defense. Exploitation of the selective toxicity of chemicals is an ancient and widely employed technique (Charles R Craig & Robert E Stitzel., 2004).

**The goals of chemotherapy**

Treatments like radiation and surgery are considered as local treatments where they act only in one area of the body such as the lung, breast or prostate and usually target the cancer directly. Chemotherapy differs from radiation or surgery in that it's almost always used as a systemic treatment. This means the drugs need to travel throughout the body to reach cancer cells wherever they are

Ther 3 possible goals for chemotherapy treatment are:

**Cure:** If possible, chemotherapy is used to cure the cancer which means that the cancer disappears and does not return. Hence, most doctors do not use the word "cure" except as a intention or possibility. Giving treatment that has a chance of curing a person's cancer, the doctor may describe it as a treatment with curative intent. But there are no guarantees and the cure may be the goal, but it doesn't always work out that way. It often takes many years to know wheather a person's cancer is actually cured.

**Control:** If cure is not possible, the goal may be to control the disease that is to shrink any cancerous tumors or stop the cancer from growing and spreading to other regions. This can help patient with cancer feel better and possibly live longer.

**Palliation:** When the cancer is at an advanced stage, chemotherapy drugs can be used to relieve symptoms that are caused by the cancer. The only goal of a certain treatment is to improve the quality of life but not treat the disease, its called palliation or palliative treatment (Gullate *et al.,* 2004).

**Chemotherapy drugs work in various ways:**

Drugs act by preventing cell division - these are known as cytotoxic drugs.

- Targeting cancer cell's food source, hormones and enzymes that they require in order to grow.
- Stopping the growth of new blood vessels that supplies to tumor. Researchers at the Johns Hopkins University School of Medicine in a study discovered how a whole class of commonly used chemotherapy drugs can destroy cancer by blocking blood vessel growth.
- Triggering suicide of cancer cells - cell suicide is known medically as apoptosis.

**Patients may receive mono therapy or combination therapy:**

- **Monotherapy** - the patient is given just one drug.

- **Combination therapy** - the patient receives more than one drug.

Which type the patient receives will depend on the kind of cancer the patient has, region which is affected as well as some other health considerations.

**Chemotherapy given at different stages**

- Neo-adjuvant therapy - if the tumor size is large the surgeon may want to shrink it before surgery. This involves some pre-operative chemotherapy or radiotherapy.
- Chemoradiation therapy - the chemotherapy is given in combination with radiation therapy/radiotherapy.
- Adjuvant therapy – the chemotherapy which is given after surgery. The chemotherapy following surgery reduces the risk of death from operable pancreatic cancer by around 30%, a UK study found.

**Cell cycle**

An understanding of cell-cycle kinetics is essential for the proper use of antineoplastic agents. Many of the most effective cytotoxic agents damage DNA and their toxicity is greater during the DNA synthetic or S phase of the cell cycle. Others, such as the taxanes and vinca alkaloids block formation of a functional mitotic spindle in M phase. Human

neoplasms that are most susceptible to chemotherapy are those with a high percentage of cells undergoing division. Similarly the normal tissues that proliferate rapidly (*e.g.,* bone marrow, intestinal epithelium and hair follicles) are most subject to damage by cytotoxic drugs, which often limits their usefulness. The slowly growing tumors with a small growth fraction (*e.g*., carcinomas of the colon or non–small cell lung cancer) often are less responsive to cycle-specific drugs. Understanding of cell-cycle kinetics and the controls of normal and malignant cell growth is crucial to the design of current therapeutic regimens and the search for new drugs

All living tissue is made up of cells. Cells reproduce and grow to replace cells that are lost through injury or normal wear and tear. The cell cycle is a series of steps where both normal cells and cancer cells go through in order to form new cells. The cell cycle has 5 phases which are labeled below using numbers and letters. Since reproduction of cells happens over and over again, the cell cycle is shown as a circle. All these steps lead back to the resting phase ($G_0$), which is the starting point. After a cell reproduces, the two new daughter cells are identical. Each cells made from the first cell can go through this cell cycle again when new cells are needed (Nordqvist C., 2009).

All cancer cells do not proliferate faster than normal cells; some cancer cells reproduce more rapidly and others are more indolent. Many anticancer drugs target rapidly-proliferating cells (both normal and cancerous cells), and these agents may act at selective or multiple sites of the cell cycle. Agents with major activity in a particular phase of the cell cycle are known as cell-cycle phase–specific agents. The antimetabolites exert their major effect during the S phase. Cell-cycle phase–specific agents may also be active to a lesser extent in other phases of the cycle. Cell-cycle phase–nonspecific agents are those with significant activity in multiple phases. The alkylating agents such as nitrogen mustard are examples.

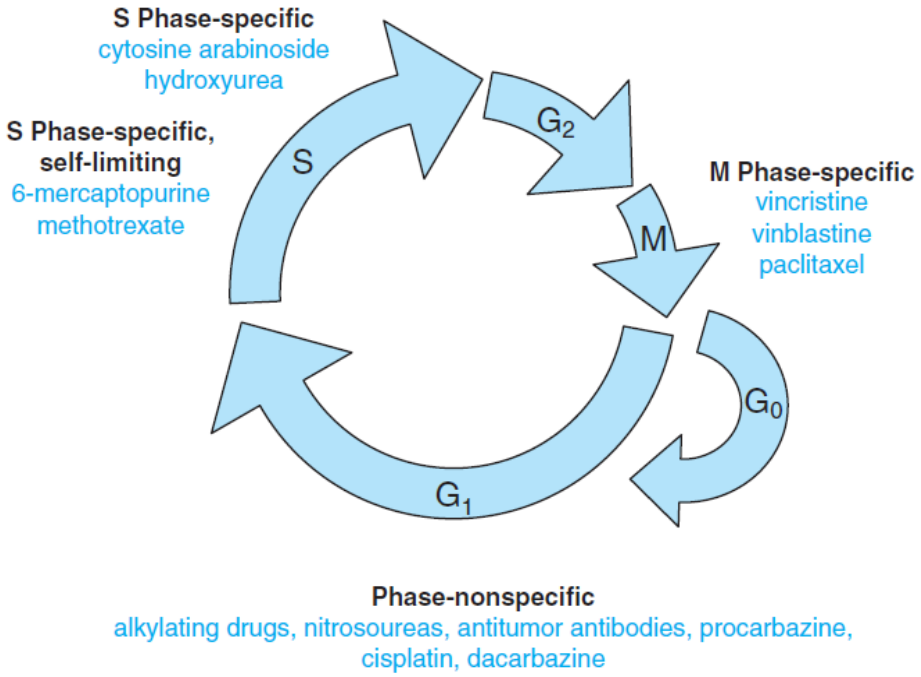



**Fig: 2 -** The cell cycle and the relationship of antitumor drug action to the cycle.

**G0 phase (resting stage):** The cell that has not yet started to divide, spend most of their lives in this phase. Depending on the type of cell, resting stage can last from a few hours to a few years. When a cell gets the signal to reproduce, it moves onto the next phase, G1.

**G1 phase:** During this phase, the cell starts making more proteins and growing larger , so the new cells will be of normal size. This G1 phase lasts about 18 - 30 hours.

**S phase:** In the S phase, the chromosomes containing the genetic code (DNA) are copied so that both of the new daughter cells formed will have strands of DNA that are matching. This phase lasts about 18 to 20 hours.

**G2 phase:** In the G2 phase, the cell checks the DNA and gets ready to start splitting into two cells. This phase lasts about 2 to 10 hours.

**M phase (mitosis):** In this phase, which lasts only 30 to 60 minutes, the cell actually splits into 2 new cells.

The cell cycle is important because many chemotherapy drugs work only on cells that are actively reproducing (not cells that are in the G0, resting phase). Some chemotherapeutic drugs specifically attack cells in a particular phase of the cell cycle (the S or M phases). Knowing how these drugs work helps oncologists to predict which drugs are likely to work well when comparing to others. Oncologists can also plan how often doses of each drug need to be given based on the timing of the cell phases.

Chemotherapy drugs cannot tell the difference between cancer cells and reproducing cells of normal tissues (those that are replacing worn-out normal cells). Because of that normal cells are damaged and this results in side effects of therapy. Each time chemotherapy is given, it involves trying to find a balance between sparing the normal cells (to lessen unwanted side effects) and destroying the cancer cells (in order to cure or control the disease).

**SIDE EFFECTS OF CHEMOTHERAPY**

Most people immediately link chemotherapy with uncomfortable side effects. However, over the last twenty years side-effect management has improved considerably. Many side effects that were once inevitable in an treatment can be well controlled or either prevented today. However, there is no reliable way to predict how patients may react to different drugs in chemotherapy. Some patients experience very mild side-effect, while some people will report various symptoms, others will have none at all.

Depending on the type of treatment and cancer, chemotherapy might have a bigger impact on the patient's work status than radiation therapy. According to researchers at the Dana-Farber Cancer-Institute women with breast cancer receiving chemotherapy appear more likely than those treated with radiotherapy to experience a change in work status. Below is a list of the most commonly reported side effects:

**Nausea and vomiting**

Over half of all patients receiving chemotherapy will experience nausea and vomiting. Doctors will usually prescribe anti-emetics. These drugs need to be taken even when symptoms have gone since they will prevent them from coming back. If the anti-emetics drugs do not work the patient should contact doctor who may switch to another anti-emetic.

**Alopecia (Hair loss)**

Some chemotherapy medications cause hair loss while others do not. If treatment starts hair does start to fall out, this will usually happen in a few weeks. In some occasions the hair will become more brittle and thinner (without falling out). In any part of the body hair loss can occur. Although hair loss has no physical health consequences, it

may cause embarrassment and distress for some people. Comparing to the men the psychological impact tends to be greater among women.

**Fatigue**

Most patients will experience some degree of fatigue on receiving chemotherapy. This may be a general feeling that exists most of the day, or only appear after certain activities. Oncologists say patients need to make sure that, not to perform tasks which are overtiring and they get plenty of rest. While light exercise has been shown to help patients and it is important to keep the activities 'light'.

**Hearing impairment (deafness, ototoxicity)**

Scientists from Oregon Health & Science University reported that deafness as a side effect of chemotherapy has long been underreported by the medical community, because a well-known classification system doctors use for reporting toxicities in patients does not consider high-frequency hearing loss, allowing the magnitude of ototoxicity (hearing damage) in children treated with platinum agents to be miscalculated.

**Neutropenia (low white blood cells) - Susceptibility to infections**

When receiving chemotherapy the immune system will be weakened because the white blood cell count will go down. White blood cells form a part of our immune system as they fight infection. Because of that, patients become more susceptible to infections. Some patients will be prescribed antibiotics which may reduce their risk of developing infections.

**Thrombocytopenia (low blood platelet count) - Blood clotting problems**

Chemotherapy may lower the patient's platelet count in blood. A platelet is a type of blood cell which helps the blood to clot (coagulate). Coagulation is essential, otherwise bleeding does not stop. Decreasing blood platelet counts linked to chemotherapy is a risk, but less severe than lower white or red blood cell counts. If patient with low platelet count will bruise more easily, patient will be more likely to have bleeding gums and nosebleeds, and if patient made a cut in a body, it may be harder to stop the bleeding. Patient's whose blood platelet counts fall too low will need a blood transfusion.

**Anemia (low red blood-cell count)**

As well as lowering in white blood cell count, chemotherapy results in lowering patient's red blood cell count. Organs and tissues inside your body get their oxygen from the red blood cells. If patient's red blood cell count goes down, too many parts of body will not get enough oxygen and will develop anemia in patients.

Anemia linked to chemotherapy requires immediate attention. To bring the red blood cell count back up immediately a blood transfusion will be done. Erythropoietin (EPO) is a drug that makes the body produce more red blood cells.

**Mucositis (inflammation of the mucous membrane)**

Chemotherapy attacks rapidly dividing cells such as bone marrow cells, blood cells, and cells of the mucous membranes that line the digestive system - this includes the mouth, esophagus, stomach, intestines and the rectum to the anus. Chemotherapy cause damage and even destroy some of those mucous membrane cells.

Oral Mucositis (in the mouth) - patients more commonly experience symptoms in their mouth. If symptoms do appear, they will usually appear about 7 to 10 days after treatment starts. The inside of the mouth, patient may feel like sunburn some people say it feels as if the area had been scalded. Ulcers often appear on the the tongue, lining of the mouth and sometimes around the lips. The rigorousness of symptoms is closely linked to the strength of the chemotherapy dose.

**Loss of appetite**

Loss of appetite is a common side effect of chemotherapy. It is possible that the cancer itself or chemotherapy, affects the body's metabolism. If appetite come back when the treatment is finished, means that the loss of appetite is just due to the chemotherapy - although this may sometimes take a few weeks. The severity of appetite and consequent weight loss depends on the type of cancer and chemotherapy treatment.

**Nails and skin**

Chemotherapy can sometimes cause sore and dry skin. Nails may also become brittle and flaky. The skin sometime become more sensitive to sunlight. It is very important to protect pateint from too much sunlight exposure. This includes staying out of the sun

during peak times of the day, make use of sun blocks and wearing clothes that provide maximum protection.

**Cognitive problems**

About one fifth of patients undergoing chemotherapy report some kind of cognitive problems including memory, attention and thinking. This sometimes can have an impact on daily tasks.

Symptoms may include:

- Shorter attention span, attention problems, concentration and focus .
- Comprehension and understand problems.
- Memory problems, especially the short-term memory.
- Organizational skills may be affected.
- Judgment and reasoning problems.
- Multitasking problems (performing/thinking about several things at the same time).
- Mood swings.

**Libido (sex drive) and fertility**

Chemotherapy may result in a lower sex drive (less interest in sex) for a significant proportion of patients. This is temporary and usually returns after treatment is completed. Chemotherapy may also damage men's sperm depending on the type of medication administered. Some women may become infertile. In most cases - though not all - fertility returns after treatment is over.

**Bowel movement problems (diarrhoea or constipation)**

There is a risk of diarrhoea sometimes when damaged cells in the intestinal tract are rapidly expelled from the body. For chemotherapy patients constipation is also a possible risk.

**Depression**

The risk of developing depression is already higher for patients with cancer. It is normal to feel anxious, distressed, stressed and sad - especially if you are concerned about what the future holds and whether treatment is going to be effective.

**Different types of chemotherapy drugs**

Chemotherapy drugs can be divided into several groups based on factors such as their chemical structure, how they work and their relationship to another drug. Some drugs act in more than one way in our body and they may belong to one or more group. Understanding how the drug works is important in predicting side effects of drug. This helps docter's to decide which drugs are likely to work well compare to other. If one or more drug are using, this information also helps them to plan exactly when each of the drugs should be given to patient (in which order and how often) (Mori T *et al.,* 2006).

**Classification of the Anticancer Drugs**

**I. Alkylating agents**

A. Nitrogen mustards

1. Mechlorethamine hydrochloride (*Mustargen,* HN2, nitrogen mustard)
2. Cyclophosphamide (*Cytoxan*)
3. Chlorambucil (*Leukeran*)
4. Melphalan (*Alkeran, L-PAM,* L-phenylalanine mustard)
5. Ifosfamide (*Ifex*)

B. Alkyl sulfonates

1. Busulfan (*Myleran*)

C. Nitrosoureas

1. Carmustine (BCNU, *BiCNU*)
2. Lomustine (CCNU, *CeeNU*)
3. Semustine (methyl-CCNU)
4. Streptozocin (*Zanosar,* streptozotocin)

D. Ethylenimines

1. Thiotepa

E. Triazenes

1. Dacarbazine (*DTIC-Dome*)

**II. Antimetabolites**

A. Folate antagonist

1. Methotrexate (*Folex, Mexate*)

B. Purine analogues

1. Thioguanine (6-TG, 6-thioguanine)

2. Mercaptopurine (6-MP, *Purinethol*)
3. Fludarabine (*Fludara*)
4. Pentostatin (deoxycoformycin, *Nipent*)
5. Cladribine (2-chloro-deoxyadenosine, *Leustatin*)

C. Pyrimidine analogues

1. Cytarabine (cytosine arabinoside, *Cytosar-U,* ara-C)
2. Fluorouracil (5-FU, 5-fluorouracil)

**III. Antibiotics**

A. Anthracyclines

1. Doxorubicin hydrochloride (*Adriamycin*)
2. Daunorubicin (daunomycin, *Cerubidine*)
3. Idarubicin (*Idamycin*)

B. Bleomycins

1. Bleomycin sulfate (*Blenoxane*)

C. Mitomycin (mitomycin C, *Mutamycin*)

D. Dactinomycin (actinomycin D, *Cosmegen*)

E. Plicamycin (*Mithracin*)

**IV. Plant-derived products**

A. Vinca alkaloids

1. Vincristine (*Oncovin*)
2. Vinblastine (*Velban*)

B. Epipodophyllotoxins

1. Etoposide (VP-16, *Vepesid*)
2. Teniposide (VM-26, *Vumon*)

C. Taxanes: paclitaxel( *Taxol*)

**V. Enzymes**

A. L-Asparaginase (*Elspar*)

**VI. Hormonal agents**

A. Glucocorticoids

B. Estrogens, antiestrogens

1. Tamoxifen citrate (*Nolvadex*)
2. Estramustine phosphate sodium (*Emcyt*)

C. Androgens, antiandrogens

1. Flutamide (*Eulexin*)

D. Progestins

E. Luteinizing hormone–releasing hormone (LH-RH) antagonists

1. Buserelin (*Suprefact*)
2. Leuprolide (*Lupron*)

F. Octreotide acetate (*Sandostatin*)

**VII. Miscellaneous agents**

A. Hydroxyurea (*Hydrea*)

B. Procarbazine (*N*-methylhydrazine, *Matulane, Natulan*)

C. Mitotane (o,p′-DDD, *Lysodren*)

D. Hexamethylmelamine (HMM)

E. Cisplatin (*cis*-platinum II; *Platinol*)

F. Carboplatin (*Paraplatin*)

G. Mitoxantrone (*Novantrone*)

**VIII. Monoclonal antibodies**

**IX. Immunomodulating agents**

A. Levamisole (*Ergamisol*)

B. Interferons

1. Interferon alfa-2a (*Roferon-A*)
2. Interferon alfa-2b (*Intron A*)

C. Interleukins: aldesleukin (interleukin-2, IL-2, *Proleukin*)

**X. Cellular growth factors**

A. Filgrastim (G-CSF; *Neupogen*)

B. Sargramostim (GM-CSF, *Leukine, Prokine*)

(Proprietary (*italics*) and other names are given in parentheses).

(Charles & Robert., 2004)

**ALKYLATING AGENTS**

Alkylating agents are compounds that are capable of introducing alkyl groups into nucleophilic sites on other molecules through the formation of covalent bonds. These nucleophilic targets for alkylation include the sulfhydryl, phosphate, amino, hydroxyl,

carboxyl and imidazole groups that are present in macromolecules and low-molecular-weight compounds within cells. In alkylating agents, the main step is the formation of a carbonium ion, which is a carbon atom with only 6 electrons in its outer shell. These type of ions are highly reactive and react instantaneously with an electron donor such as an amine, sulfhydryl or hydroxyl group. Most of the cytotoxic anticancer alkylating agents are bifunctional in action, i.e. they have two alkylating groups.

The most common binding site for alkylating agents is the seven-nitrogen group of guanine. These covalent interactions result in cross-linking between two DNA strands or between two bases in the same strand of DNA. Reactions between DNA and RNA and between drug and proteins may also occur, but that results in cell death because the interlinked strands do not separate as required. Because the alkylating agents can damage DNA during any phase of the cell cycle, they are not cell-cycle–phase specific. However, their greatest effect is seen in rapidly dividing cells. As a class alkylators are cytotoxic, carcinogenic, mutagenic, teratogenic and myelosuppressive. Resistance to these agents can occur from increased DNA repair capabilities, from accelerated exit from or decreased entry into cells, from increased inactivation of the agents inside cells, or from lack of cellular mechanisms to result in cell death following DNA damage. They react with water and are inactivated by hydrolysis, making spontaneous degradation an important component of their elimination (Morrow and Harris ., 2000).

**Toxicities of Alkylating Agents**

- **Bone Marrow Toxicity**

The alkylating agents differ in their patterns of antitumor activity and in the sites and severity of their side effects. Most of them cause dose-limiting toxicity to bone marrow elements and intestinal mucosa (to a lesser extent). Most alkylating agents including chlorambucil, nitrogen mustard, melphalan, cyclophosphamide and ifosfamide cause acute myelosuppression with a lower level of the peripheral blood granulocyte count at 6 - 10 days and recovery in 14 - 21 days. Cyclophosphamide has lesser effects on peripheral blood platelet counts than do the other agents. Carmustine and other chloroethylnitrosoureas cause delayed and prolonged suppression of both granulocytes and platelets, reaching the low value 4 to 6 weeks after drug administration and reversing slowly thereafter. Busulfan suppresses all blood elements, particularly stem cells and may

produce a prolonged and cumulative myelosuppression lasting months or even years. For this reason, it is used as a preparative regimen in allogenic bone marrow transplantation.

Both humoral and cellular immunity are suppressed by alkylating agents, which have been used to treat various autoimmune disorders. Immunosuppression can be reversible at doses used in most anticancer protocols.

- **Mucosal Toxicity**

In addition to effects on the hematopoietic system, alkylating agents are highly toxic to dividing mucosal cells, leading to oral mucosal ulceration and intestinal stripping. In high-dose chemotherapy protocols associated with bone marrow reconstitution, the mucosal effects are particularly significant, as they predispose to bacterial sepsis arising from the gastrointestinal tract. In these protocols, cyclophosphamide, thiotepa and melphalan have the advantage of causing less mucosal damage than the other agents. In high-dose protocols, however a number of additional toxicities become limiting.

- **Neurotoxicity**

CNS toxicity is manifest in the form of nausea and vomiting, particularly after intravenous administration of nitrogen mustard or BCNU. High-dose busulfan may cause seizures; in addition, it accelerates the clearance of an antiseizure medication, phenytoin. Ifosfamide is the most neurotoxic of this class of agents, producing altered mental status, generalized seizures, coma and cerebellar ataxia. These side effects have been linked to the release of chloroacetaldehyde from the phosphate-linked chloroethyl side chain of ifosfamide.

- **Other Organ Toxicities**

While mucosal and bone marrow toxicities occur predictably and acutely with conventional doses of these drugs, other organ toxicities may occur after prolonged or high-dose use; these effects can appear after months or years of therapy and may be irreversible and even lethal. Usually after several months of treatment all alkylating agents causes pulmonary fibrosis. Particularly those employing busulfan or BCNU in high-dose regimens, vascular endothelial damage may result in veno-occlusive disease (VOD) of the liver, which is an fatal side effect that is successfully reversed by the investigational drug defibrotide (Richardson., 2003). The ifosfamide and nitrosoureas

after multiple cycles of therapy may lead to renal failure. Cyclophosphamide and ifosfamide release a urotoxic and nephrotoxic metabolite called acrolein, which causes a severe hemorrhagic cystitis. Ifosfamide in high doses for transplant causes a chronic and often irreversible renal toxicity. Proximal and less commonly distal tubules may be affected, with difficulties in $Mg^{2+}$ and $Ca^{2+}$ reabsorption, renal tubular acidosis and glycosuria. Nephrotoxicity can be correlated with the total dose of drug received and increases in frequency in children less than 5 years of age. The syndrome has been attributed to acrolein and/or chloroacetaldehyde excreted in the urine (Skinner, 2003).

The more unstable alkylating agents (particularly mechlorethamine and the nitrosoureas) have strong vesicant properties, damage veins with repeated use and if extravasated, produce ulceration. Most alkylating agents cause alopecia. Alkylating agents have toxic effects on the female and male reproductive systems, causing an often permanent amenorrhea, particularly in perimenopausal women, and an irreversible azoospermia in men.

- **Leukemogenesis**

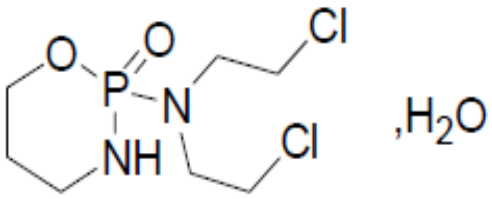
As a class of drugs, the alkylating agents are highly leukemogenic in nature. Acute nonlymphocytic leukemia, often associated with partial or total deletions of chromosome 5 or 7, peaks in incidence about 4 years after therapy and may affect up to 5% of patients treated on regimens containing alkylating drugs (Levine and Bloomfield., 1992). It often is preceded by a period of anemia or neutropenia and bone marrow morphology consistent with myelodysplasia. Methylating agent procarbazine and Melphalan, the nitrosoureas have the greatest propensity to cause leukemia, where as it is less common with cyclophosphamide.

## CLINICAL PHARMACOLOGY

### Nitrogen Mustards

Nitrogen mustards are related to the 'mustard gas' used during the First World War; their basic formula (R-*N*-*bis*-(2-chloroethyl)). In the body, each 2-chloroethyl side-chain undergoes an intramolecular cyclisation with the release of a $Cl^-$. The highly reactive ethylene immonium derivative so formed can interact with DNA and other molecules

**CYCLOPHOSPHAMIDE**




Cyclophosphamide Monohydrate

**Physico –chemical properities**

**Table : 1-** Physio-chemical properties of cyclophophamide

| | |
|---|---|
| Molecular Formulae | $C_7H_{15}C_{12}N_2O_2P.H_2O$ |
| Chemical Name | 2H-1,3,2-Oxazaphosphorin-2- amine,N,N-bis (2-chloroethyl) tetrahydro,2- oxide, monohydrate |
| Colour | fine white crystalline powder. |
| Odour | Odourless |
| solubility | soluble in water, saline, ethanol. |
| Melting point | 49□C-51□C |
| Storage temperature | 2□C- 8□C |
| Water solubility | 40g/L |
| Sensitivity | Light and moisture sensitive |
| Hazard Class | 6.1 |

Cyclophosphamide is probably the most commonly used alkylating agent. Cyclophosphamide administration results in the formation of cross-links within DNA due

to a reaction of the two chloroethyl moieties of cyclophosphamide with adjacent nucleotide bases. Cyclophosphamide must be activated metabolically by microsomal enzymes of the cytochrome P450 system before ionization of the chloride atoms and formation of the cyclic ethylenimmonium ion can occur. It is inactive until metabolised in the liver by the P450 mixed function oxidases . It has a pronounced effect on lymphocytes and can also be used as an immunosuppressant. It is usually given orally or by intravenous injection but may also be given intramuscularly.

**Mechanism of action**

Cyclophosphamide undergoes metabolism to several intermediates with alkylating activity. The principal metabolites identified are phosphoramide mustard and acrolein. Phosphoramide mustard can undergo dephosphoramidation to yield nornitrogen mustard, which also has alkylating activity. Metabolites of cyclophosphamide can interact with proteins and DNA, resulting in the formation of adducts. A minor pathway results in dechloroethylation and the formation of 2-dechloroethylcyclophosphamide and another alkylating agent, chloroacetaldehyde (Balu *et al.,* 2002). The other compounds such as 4-ketocyclophosphamide and propionic acid derivative are relatively non-toxic and are the major urinary metabolites of cyclophosphamide in several species (IARC., 1981).

Important toxic effects are nausea, vomiting, haemorrhagic cystitis and bone marrow depression. This last effect is caused by the metabolite acrolein and can be ameliorated by increasing fluid intake and administering compounds that are sulfhydryl donors, such as mesna (sodium-2-mercaptoethane sulfonate) or N-acetylcysteine. These compounds interact specifically with acrolein, forming a non-toxic compound.

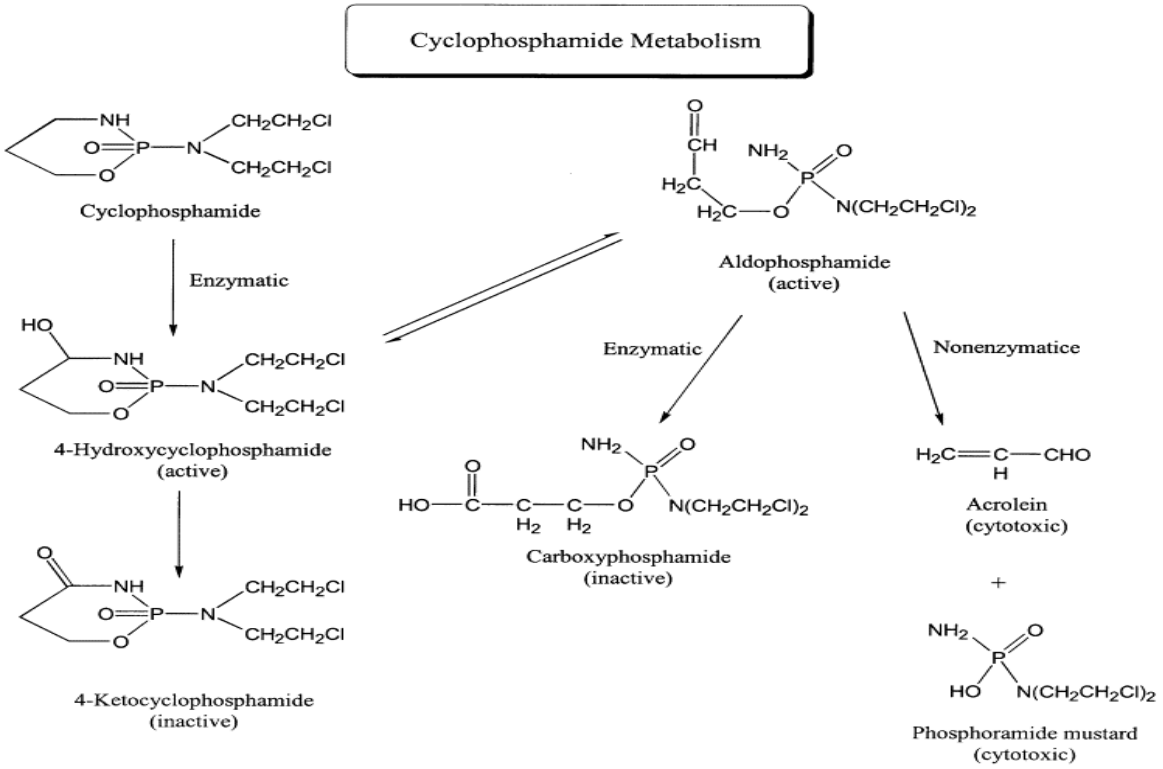



**Fig : 3-**Metabolism of cyclophosphamide ( Rang *et al*., 2007).

**Single-Agent Dosage**

3.5–5 mg/kg/d orally for 10 days; 1 g/m$^2$ IV as single dose

**Major uses**

Cyclophosphamide is used in the treatment of chronic lymphocytic leukaemia, lymphomas, soft tissue and osteogenic sarcoma, and solid tumours. It is given orally or intravenously.

(a) Hodgkin lymphoma

(b) Non-Hodgkin lymphoma

(c) Multiple myeloma

(d) Leukaemia

(e) Cutaneous T-cell lymphoma

(f) Neuroblastoma

(g) Cancer of the ovary

(h) Retinoblastoma

(i) Cancer of the breast

(j) Small cell cancer of the lung

(k) Sarcoma

**Major side effects**

Hemorrhagic cystitis

Moderately emetogenic: worse with high doses;

Nausea may be delayed 12–24 hours;

Myelosuppression;

Alopecia;

SIADH, typically with high doses (>2 $g/m^2$);

Secondary malignancies (bladder cancers, acute leukemia);

Infertility, sterility.

**CHEMOPROTECTIVE AGENTS**

Chemoprotective agents are the drugs that are used with certain types of chemotherapy to protect the body from or minimize the side effects of the chemotherapy. Chemoprotective agents include amifostine, mesna and dexrazoxane.

Amifostine – Approved by the FDA in 1985, amifostine helps reduce the level of renal injury in some cancer patients treated with chemotherapy. Amifostine prevents cyclophosphamide (DNA-binding drugs), as well as drugs like cisplatin( which contain platinum) from binding to cells. Amifostine actually makes use of extracellular conditions such as higher pH, alkaline phosphatase activity and better vascular delivery of the drug to normal tissue, in order to protect normal tissue, while not extending this same type of defense to cancer cells. Amifostine is not an active drug on its own and instead is converted into an active metabolite inside cells.

Mesna – Approved by FDA in 1988, mesna is used to decrease bladder irritation (hemorrhagic cystitis) caused by certain high-dose chemotherapy protocols. In general these medications do not eliminate side effects. Instead, they protect the body from some of the potentially serious side effects. These drugs also posses side effects of their own so they are used only with specific types of chemotherapy treatment or when the benefit clearly is greater than the risk.

Dexrazoxane – Approved by the FDA in 1995, use of dexrazoxane has resulted in a significant decrease in cardiac events in cancer patients undergoing certain chemotherapy treatments. Dexrazoxane is another such chemoprotective drug which is particularly well-suited to keep anthracycline antibiotics from damaging cardiac tissue. Anthracyclines can able to disrupt certain receptors in heart tissue, cause accumulations of cytotoxic metabolites and promote free radical formation, which is why these drugs are not used to treat normal infections, despite being antibiotics.

Today's chemoprotective drugs which are usually administered with a specific type of chemotherapy drug; for example, Mesna which is used along with oxazophosphorines class of drugs. Mesna binds to the toxic metabolites of this class of chemotherapy drugs, helping to prevent urogenital problems and bladder inflammation.

Another chemoprotective drug used to reduce the side effects of cisplatin is N-Acetylcysteine(NAC).In clinical trials, N-Acetylcysteine given up to 2 hours after administration of cisplatin, it reversed cytotoxic damage to tumor cell lines and prevented programmed cell death. N-Acetylcysteine seems to prevent cisplating binding mitochondrial and cellular receptors which normally begin the process of apoptosis. Since this drug(N-Acetylcysteine) does not cross the blood-brain barrier, it could be used in patients with brain cancer to protect many organs from damage without reducing the tumor-killing properties of the cisplatin.

Chemoprotective agents are still a new field of study and research will likely yield agents with greater selectivity for normal tissue, as well as the ability to protect a larger number of tissue types. Nanoparticle delivery may also assist in minimizing the side effects of chemotherapy.

**OXIDATIVE STRESS**

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include peroxides and free radicals. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds

(including quinones) into more aggressive radical species that can cause extensive cellular damage (Valko M *et al*., 2005).

Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases including hypertension, atherosclerosis, ischemic diseases, diabetes mellitus and malignancies. Oxidative stress can be defined as harmful because oxygen free radicals attack biological molecules such as DNA, lipids and proteins. However, oxidative stress also has a useful role in physiologic adaptation and in the regulation of intracellular signal transduction. It can cause disruptions in normal mechanisms of cellular signaling. Therefore, a more useful definition of oxidative stress may be a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them. The various biomarkers that can be used to assess oxidative stress in vivo have been attracting interest of scientists because of the accurate measurement of such stress is necessary for investigation of its role in lifestyle diseases as well as to evaluate the efficacy of treatment. Many markers of oxidative stress have been proposed, including lipid 4-hydroxynonenal, hydroperoxides, isoprostan, ubiquinol-10 and 8-hydroxyguanine.

Oxidative stress not only has a cytotoxic effect, but also plays an important role in the modulation of messengers that regulate essential cell membrane functions, which are vital for survival. It affects the intracellular redox status, leading to the activation of protein kinases, including a series of receptor and non-receptor tyrosine kinases, protein kinase C and the MAP kinase cascade and hence induces various cellular responses. These protein kinases play an important role in cellular responses such as activation, proliferation and differentiation, as well as various other functions. Oxidative stress can influence many biological processes such as apoptosis, inflammatory reactions and viral proliferation. In these processes, gene transcription factors such as nuclear factor-_B (NF-_B) and activator protein-1 (AP-1) act as oxidative stress sensors through their own oxidation and reduction cycling. This type of chemical modification of proteins by oxidation and reduction is called reduction-oxidation (redox) regulation (Toshikazu Yoshikawa and Yuji Naito., 2002).

Oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, glomerulonephritis, vasculitis, lupus erythematosus, adult respiratory distress syndrome), ischemic diseases (stroke, heart disease, intestinal ischemia),

hemochromatosis, acquired immunodeficiency syndrome (AIDS), emphysema, organ transplantation, hypertension, gastric ulcers and preeclampsia, neurologic diseases (Alzheimer's disease, Parkinson disease, multiple sclerosis, amyotrophic lateral sclerosis), muscular dystrophy, smoking-related diseases, alcoholism and many others. The reason that overproduction of free radicals is a feature of such a broad spectrum of diseases derives from the fact that oxidative metabolism is a necessary part of every cell's metabolism. If a cell that is sick or injured in any way that results in mitochondrial injury (leaky membranes, calcium influx and so forth), then increased production of superoxide is likely to result (Joe M Mc Cord *et al*., 2000).

Reactive Oxygen Species are generated by a number of pathways. Most of the oxidants that are produced by cells occur as:

- Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, thereby foreign proteins (antigens) are denatured.
- A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.
- Xenobiotic metabolism, i.e., detoxification of toxic substances.

**ANTI OXIDANTS**

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can result in the production of free radicals and these radicals can start chain reactions. When the chain reaction starts in a cell, it can cause damage or death to the cell. Antioxidants terminate these type of chain reactions by inhibiting other oxidation reactions and removing free radical intermediates. They do this by being oxidized themselves, so antioxidants are often reducing agents such as ascorbic acid, asthiols or polyphenols (Sies and Helmut., 1997). Antioxidants are capable of stabilising or deactivating free radicals before they attack cells and are critical for maintaining optimum cellular and systemic health and well being .There are mainly three types of antioxidants.

**A: Enzymes**

Antioxidant enzymes are antioxidants that can be uniquely synthesized in the human body (eg. Superoxide dismutase, catalase and peroxiredoxins). They are made from the protein and minerals in the food we eat.

SOD is present in both inside and outside cell membranes, it is one of the body's primary internal anti-oxidant defenses and plays a important role in reducing the oxidative stress implicated in atherosclerosis and other life-threatening diseases. Humans have three genes encoding superoxide dismutases (SOD), which localize in the mitochondria, the cytosol, or the extracellular spaces. These genes are derived from two ancestral genes. One gene gave rise to the copper-and-zinc– containing enzymes; the other gave rise to the manganese- or iron containing enzymes. The SODs catalyze the reaction:

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \longrightarrow 3H_2O_2 + O_2$$

This dismutation or disproportionation reaction makes use of the fact that superoxide is both an oxidant and a reductant, eager to get rid of its extra electron or to take on another electron. The enzyme uses one superoxide radical to oxidize another. Catalases work in much the same way, because hydrogen peroxide can be a weak reductant as well as a fairly strong oxidant. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani P *et al*., 2004).

$$H_2O_2 + H_2O_2 \longrightarrow H_2O + O_2$$

In higher organisms, glutathione peroxidases appear to have largely supplanted the need for catalase. These enzymes use NADPH as the reducing species for hydrogen peroxide:

$$NADPH + H^+ + H_2O_2 \longrightarrow 2H_2O + NADP^+$$

They can reduce lipid peroxides as well as hydrogen peroxide and are very important enzymes in the prevention of lipid peroxidation to maintain the structure and function of biologic membranes. Glutathione peroxidise act as catalysts in the liver facilitating reactions that render toxins less harmful (Umulis D M *et al*., 2005).

**B: Vitamins**

Vitamins cannot be created in our bodies. It need to be supplemented every day without any fail. The typical antioxidant vitamins are vitamins A, C, E, and M (folic acid) etc.

**C: Phytochemicals**

Phytochemicals are antioxidants that are present in many plants to protect themselves and it has been found that if people ingest these plants the phytochemicals present in it work as antioxidants within our bodies, eg: blue berry, tomato, spinach.

The body relies on several endogenous defense mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes – glutathione peroxidase, catalase and superoxide dismutase (SOD) – metabolize oxidative toxic intermediates and require micronutrient cofactors such as iron, selenium, zinc, copper and manganese for optimum catalytic activity. Inadequate dietary intake of these trace minerals has been suggested that they may compromise the effectiveness of these antioxidant defense mechanisms (Duthie and Brown .,1994). Glutathione is an important water-soluble antioxidant which is synthesized from the amino acids glutamate, glycine and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. Exposure of the liver to xenobiotic substances induces oxidative reactions through the upregulation of detoxification enzymes, i.e., cytochrome P-450 mixed-function oxidase. When an individual is exposed to high levels of xenobiotics, more glutathione is utilized for conjugation (a key step in the body's detoxification process) making it less available to serve as an antioxidant. Research suggests that glutathione and vitamin C work interactively to quench free radicals and that they have a sparing effect upon each other (Jacob ., 1995).

**PLANTS AS ANTIOXIDANTS**

In search of novel sources of antioxidants in the last years, medicinal plants have been extensively studied for their antioxidant activity. From ancient times, herbs have been used in many areas including nutrition, medicine, beverages, cosmetics, flavoring etc. The ingestion of fresh vegetables, fruit and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases (Willcox *et al.*, 2004). The higher intake of plant foods correlates with lower risk of mortality from these diseases (Johnson., 2001). Approximately 60 % of the commercially available anti-tumoral and antiinfective agents are of natural origin (Cragg *et al.*, 1997).

Many plant-derived substances, collectively termed "phytochemicals" or "phytonutrients" are increasingly known for their antioxidant activity. Botanicals have been used for treatment or prevention of various human diseases throughout history. The

cancer chemopreventive activities of naturally occurring phytocompounds is of great interest. Many indigenous herbal plants of regional interest have been used popularly as folk medicines in India or other Asian countries.

Cancer chemoprevention by using antioxidant approaches has been suggested to offer a good potential in providing important fundamental benefits to public health and it is now considered by many oncologists and researchers as a key strategy for delaying, inhibiting, or even reversal of the process of carcinogenesis (Lie-Fen Shyur *et al.,* 2005). Plants having vitamins (C, E, carotenoids, etc.), flavanoids (flavones, isoflavones, flavonones,anthocyanins and catechins), polyphenols (ellagic acid, gallic acid and tannins) possess remarkable anti oxidant activity. Anti oxidant activity is neither restricted to a particular part of the plant nor the specific families (Vivek and Surendra ., 2006).

Besides, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects such as free radical scavenging abilities, antioxidant, anti- inflammatory, anticarcinogenic etc. As crude extracts of herbs and spices and other plant materials that are rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. While, flavonoids are a group of polyphenolic compounds with known properties, which include inhibition of hydrolytic and oxidative enzymes, free radical scavenging and anti-inflammatory action.

Natural antioxidants especially phenolics and flavonoids from tea, wine, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or as nutritional supplements. Also many other plant species have been investigated in the search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, considerable attention in recent years has been directed towards the identification of plants with antioxidant ability (Vinay R P *et al.,* 2010).

The present study aims at evaluating the chemoprotective efficacy of the Plant *Gmelina arborea* against cyclophosphamide induced toxicity, as the well known cancer treating drug has number of side effects especially toxicity towards hematopoetic system.

# 2. REVIEW OF LITERATURE

**Anticancer activity of cyclophosphamide**

Yasmine S Touil *et al*., 2006 studied the antiangiogenic and antitumour activity of the combination of the natural flavonoid fisetin and cyclophosphamide in Lewis lung carcinoma-bearing mice. Fisetin was administered i.p. at 223 mg/kg daily for 5 days in week 1 (days 4 to 8 post tumour implantation), followed by 3 injections on $11^{th}$, $12^{th}$ and $14^{th}$ day. Fisetin treatment led to a 67% tumour growth inhibition compared to the controls. Low dose cyclophosphamide was administered at 30 mg/kg on four days in week 1 only (days 4, 5, 7, 8), and led to a tumour growth inhibition of 66%, similar to fisetin treatment. When fisetin and cyclophosphamide were combined at the same dose schedules and levels as used above, results in dramaticall declination in tumour volumes, ie 92% inhibition when compared to controls on day 15. Over the two week treatment, only a 4.6% loss in body weight was observed showing drug combination was not toxic.

Colleoni M *et al*., 2001 studied the effect of low dose oral methotrexate and cyclophosphamide in metastatic breast cancer, its antitumour activity and correlation with vascular endothelial growth factor levels (VEGF). They evaluated the clinical efficacy by means of direct cytotoxicity on tumour cells. The continous low dose CTX and MTX is minimally toxic and effective in pretreated breast cancer patients and there is a drop in VEGF which was associated with the treatment. Combinations of therapy that inhibit angiogenesis plus cytotoxic therapy may be more effective than either type of therapy alone.

**Cyclophosphamide toxicity**

Haubitz M (2007) carried out a study to find out the acute and long term toxicity of cyclophosphamide. Cyclophosphamide has been shown to increase the incidence of malignancies e.g. of the bladder, the haematopoetic system and the skin. There is a 1.6- to 2.4-fold overall increase in malignancies, depending on the total CTX dose and the time of follow-up. For skin cancer the risk increases up to 10.4-fold, for lymphomas up to 11-

fold and for leukaemia up to 5.7-fold. Regarding bladder carcinoma, the incidence varies between less than 1 %, 3 % and 5 % corresponding to a 5 to 33 fold increase. The risk rises with the total CTX dose administered and the follow-up, and an incidence of 16 % has been reported after 15 years. It is difficult to pinpoint a critical dose. Regarding patients with renal involvement or with alveolitis or other severe manifestations, oral CTX can be replaced safely by intravenous pulse administration, leading not only to a reduction of about 60 % in total dose but also to a marked risk reduction concerning leukopenia and other infections.

George B McDonald *et al.,* 2003 examined patients with hematologic malignancy undergoing allogeneic transplantation following a preparative regimen of cyclophosphamide (CTX) plus total body irradiation to study liver toxicity after cyclophosphamide and total body irradiation and concluded that increased exposure to toxic metabolites of cyclophosphamide leads to increased liver toxicity

Tatiane Yumi Nakamura Kanno *et al.,* 2009 evaluated the effect of different doses of cyclophosphamide on male mice reproductive parameters. During the study, it was observed a decrease in body mass and a decrease in kidneys and testicles weight in all animals treated with cyclophosphamide. Only those groups that received the doses 100 and 150 mg.kg-1 of cyclophosphamide were able to fertilize their females. Abnormal spermatozoa were found in the doses 200, 250 mg.kg-1.

**Chemoprotecive effect against cyclophosphamide induced toxicity**

In 1996 Kemp G *et al* studied the protective effect of Amifostine against cyclophosphamide-induced and cisplatin-induced toxicities without reducing the anticancer efficacy of these drugs: in patients with advanced ovarian cancer. Two hundred forty-two patients with advanced ovarian cancer were randomized to receive six cycles of cyclophosphamide (1,000 mg/m2) and cisplatin (100 mg/m2) with or without amifostine (910 mg/m2) every 3 weeks for six cycles. Pretreatment with amifostine before each cycle of chemotherapy resulted in a reduction of cumulative hematologic, neurologic and renal toxicities associated with the CTX regimen, with no reduction in antitumor efficacy.

Renato Santos-Mello *et al*., 2005 studied the ability of Cysteamine or 2-mercaptoethylamine (MEA) to protect mouse bone marrow polychromatic erythrocytes against the induction of micronuclei by alkylating agents such as cyclophosphamide (CTX) and methyl methanesulfonate (MMS). They observed that MEA administered intraperitoneally 30 min before or 30 min after the administration of CTX or MMS significantly reduced the frequency of micronucleated polychromatic erythrocytes (MNPCEs) induced by the alkylating agents. When Cysteamine was administered in combination with CTX or MMS the reduction in the frequency of MNPCEs did not reach levels that are statistically significant, even though it has reached the values close to significance. With respect to the polychromatic erythrocyte/normochromatic erythrocyte (PCE/NCE) ratio, they observed that MEA did not provide significant protection against the bone marrow toxicity induced by CTX.

Adel RA Abd-Allah *et al*., 2004 reported the effect of taurine, on cyclophosphamide (CTX) induced urinary bladder toxicity was investigated by administration of a single dose of CTX (150 mg/kg, i.p.). The results of their study suggest that CTX induced cystitis is related to oxidative stress and ROS formation owing to depletion of GSH and/or inflammation. Taurine, which was useful for the amelioration of the toxicity of other models of oxidative stress including hepatitis, liver ailments and the complications of diabetes can also protect the structure and function of the urinary bladder against CTX-induced oxidative stress.

Praveen Kumar *et al*., 1995 reported that oral administration of Septilin protects mice from cyclophosphamide-induced leukopenia. Total leukocyte counts in Septilin treated animals on day 12 were 2400 cells/$mm^3$ which was significantly higher ($p<0.005$) compared to controls (700 cells/$mm^3$) and reached the base line value on $16^{th}$ day (12,000 cells/$mm^3$). The bone marrow cellularity(BMC) was also significantly higher compared to controls and there was no change in the level of hemoglobin in Septilin treated animals. Septilin treatment reduced the mortality in mice and prevented the loss of body weight treated with cyclophosphamide. This indicates the usefulness of Septilin during chemotherapy-induced leukopenia.

**Plants as chemoprotectors**

Ali Shalizar Jalali *et al.,* 2011 conducted a study to assess whether *Crataegus monogyna* fruits aqueous extract with anti-oxidant properties could serve as a protective agent against reproductive toxicity during cyclophosphamide treatment in a rat model. The CTX-treated group showed significant decreases in the body and organ weights and spermatogenic activities as well as many histological alterations. CTX treatment also caused a significant decrease in sperm count and motility with an increase in abnormal and dead sperms. Significant decrease in serum levels of testosterone and increased serum concentrations of FSH, LH, LDH, CPK and SGOT were also observed in CTX-treated rats. Notably, *Crataegus* coadministration caused a partial recovery in above parameters. These findings indicate that *Crataegus monogyna* fruit extract might be partially protective against CTX-induced reproductive toxicity.

Kamel Rouissi *et al*., 2012 investigated the effects of pre-treatment with *Aloe vera* plant extract (AVE) on the urotoxicity induced by acute doses of Cyclophosphamide and Buthionine Sulfoximine using a Swiss albino mice model. The toxicity modulations were evaluated by measuring hydrogen peroxide production ($H_2O_2$), lipid peroxidation (LPO) and antioxidants in the urinary bladder of the animals. The findings revealed that *Aloe vera* plant extract induced remarkable protective effects in terms of both LPO and enzymatic antioxidant activities.

There are number of studies have been carried out using plants as chemoprotecting agents against cyclophosphamide induced toxicity. The immunomodulatory and chemoprotective effect of methanolic extract of *Acacia nilotica* (Ahmad S *et al*., 2012) and *Moringa oleifera* Lam (Anamika Gupta *et al.,* 2010) was reported. Chemoprotective effect of garlic extract towards cyclophosphamide toxicity in mice was studied by Unnikrishnan M C *et al*., 1990. In 2012, Vinod Prabhu V and Guruvayoorappan C evaluated the immunostimulant activity and chemoprotective effect of mangrove *Rhizophora apiculata* against cyclophosphamide induced toxicity in BALB/c mice. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of *Decalepis hamiltonii* in mice was studied by Mahsa Zarei and Shivanandappa T., 2013.

The effect of Unani Formulation *(Jawarish amla sada)* on Cyclophosphamide induced Toxicity in Tumour Bearing Mice was evaluated by Firoz Ahmad *et al*., 2012.

Yukmijihwang-tang (YJT) is a multi-herbal medicinal formula that has been used in traditional Asian medicine to treat male reproductive problems. Oh *M S et* al., 2007 investigated the effects of YJT on Cyclophosphamide (CTX) induced reproductive toxicities in rat testes. The results of their study suggested that YJT has a protective effect against CTX-induced reproductive toxicities by inhibiting the increases in lipid peroxidation and enhancing cAMP-responsive element modulator (CREM) expression.

Haque R *et al*., 2003 investigated the modulatory effects of walnut extract on the toxicity of an anticancer drug, cyclophosphamide (CTX) with special reference to protection against disruption of drug metabolizing and antioxidant enzymes. Plant extract+CTX group animals showed restoration in the level of cytochrome P450 (CYP) content and in the activities of glutathione S-transferase (GST), glutathione peroxidase (GPx) and catalase (CAT) in both liver and kidneys.

**Pharmacological activities of the plant *Gmelina arborea*.**

Rezvanfar M *et al*., 2008 reported that Satureja khuzestanica essential oil (SKEO) protects reproductive system of rats from toxicity of cyclophosphamide through its antioxidant potential and androgenic activity *Gmelina arborea*.

Anuj M Pandey & Yogesh Kulkarni (2010) studied the antioxidant activity of bark of *Gmelina arborea* by various *In vitro* techniques. Phytochemical studies were performed on the extracts ie, Methanolic extract (ME) and Aqueous extract (AE). Assays performed were Free radical scavenging assay, Reducing power assay and Nitric oxide screening activity. The study revealed that the plant *Gmelina arborea* possesses antioxidant activity. The antioxidant activities of *Gmelina arborea* were attributed to the presence of phenolic compounds.

Vijay T *et al*., 2011 investigated the potential protective effect of *Gmelina arborea* (GA) against Doxorubicin (DOX) induced cardiotoxicity in rats. GA protected against DOX-induced increased levels of marker enzymes. It significantly inhibited DOX-

provoked glutathione (GSH) depletion in cardiac tissues. The declination of cardiac activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were significantly mitigated. Pretreatment of GA significantly guarded against DOX-induced rise of serum lactate dehydrogenase (LDH). GA alleviated histopathological changes in rats' hearts treated with DOX. In conclusion, GA protects against DOX-induced cardiotoxicity in rats. The results can be attributed to GA's antioxidant activity.

Syamsul Falah *et al*., 2008 extracted the bark meal of *Gmelina arborea* Roxb. with acetone and methanol. Fractionation of the acetone extract with *n*-hexane, ethoxy ethane, ethyl acetate and subsequent chromatographic separation of the fractions led to the isolation of 4 compounds. The diethyl ether-soluble fraction yielded tyrosol [2-(4-hydroxyphenyl)ethanol], 8-5′ neolignan and (+)-balanophonin with opposite optical rotation to known gmelinol, a known lignin and (−)-balanophonin. The ethyl acetate-soluble fraction afforded a new phenylethanoid glycoside to the best of our knowledge, which was identified as (−)-*p*-hydroxyphenylethyl[5‴-*O*- (3,4-dimethoxycinnamoyl)-b-D-apiofuranosyl(1‴ → 6′)]-b-D-glucopyranoside. From the methanol extract, two known compounds, 2,6-dimethoxy-*p*-benzoquinone and 3,4,5-trimethoxyphenol , were isolated and identified. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay of the identified compounds indicated that 3,4,5-trimethoxyphenol exhibited moderate activity.

A M El-Mahmood *et al*., 2010 monitored antimicrobial activity using the agar diffusion method. He reported that the crude extracts of the leave and stem bark of the plant *G. arborea* inhibited the growth of such recalcitrant pathogenic *Klebsiella pneumonieae, Escherichia coli, Shigella dysenteriea, Proteus mirabilis,* and *Salmonella typhi.*

Ambujakshi H R *et al.,* 2009 studied the effect of anthelmentic activity of alcoholic and aqueous leaves extracts of *Gmelina arborea* Roxb. The plant extract exhibited anthelmentic activity in dose dependent manner giving shortest time of paralysis and death compared to piperazine citrate especially with 100mg/ml concentration for *Pheretima posthuma* and *Ascardia galii* worms by increasing chloride ion conduction of

worm muscle membrane produces hyperpolarisation and reduces excitability that leads to muscle to relaxation and flaccid paralysis.

Saravani P *et al.,* 2011 reported that, *Gmelina arborea* methanolic extract have significant diuretic activity on albino rats. There was an increase in the ratio of concentration of excreted sodium and potassium ions after methanolic extract of GA treatment. This indicates that the GA extract increases sodium excretion to larger extent than potassium which leads to hyperkalaemic side effect.

Pattanayak P *et al*., 2011 conducted a study to find out the effect of antidiabetic activity of ethanolic extract of *G.arborea* bark at dose of 420 mg.kg-1 and chlorpropamide at dose of 200 mg.kg-1 was found to reduce the increase of blood sugar in sreptozotocin (50 mg.kg-1) induced diabetes due to the increased blood GSH levels reinforcing the role of GSH as free radical scavenger and in the repair of free radical caused biological damage.

# 3. AIM

In recent years much attention has been showered on the therapeutic impact of plant derived drugs and phytochemicals on various infectious and non infectious diseases. Compared to its alternative forms, herbal medicine has high efficacy, is economically cheaper and imposes lesser side effects on the subject. The selection of this plant *Gmelina arborea* was made on the basis of its:

- High therapeutic value
- Wide use in Ayurvedic System of Medicine
- Degree of research work which is not yet performed

Very less pharmacological studies have been carried out on the barks of *Gmelina arborea*. Hence, we have decided to submit the project on *Gmelina arborea* emphasizing on the chemoprotective activity against cylcophosphamide induced toxicity.

# 4. SCHEME OF THE PROPOSED WORK

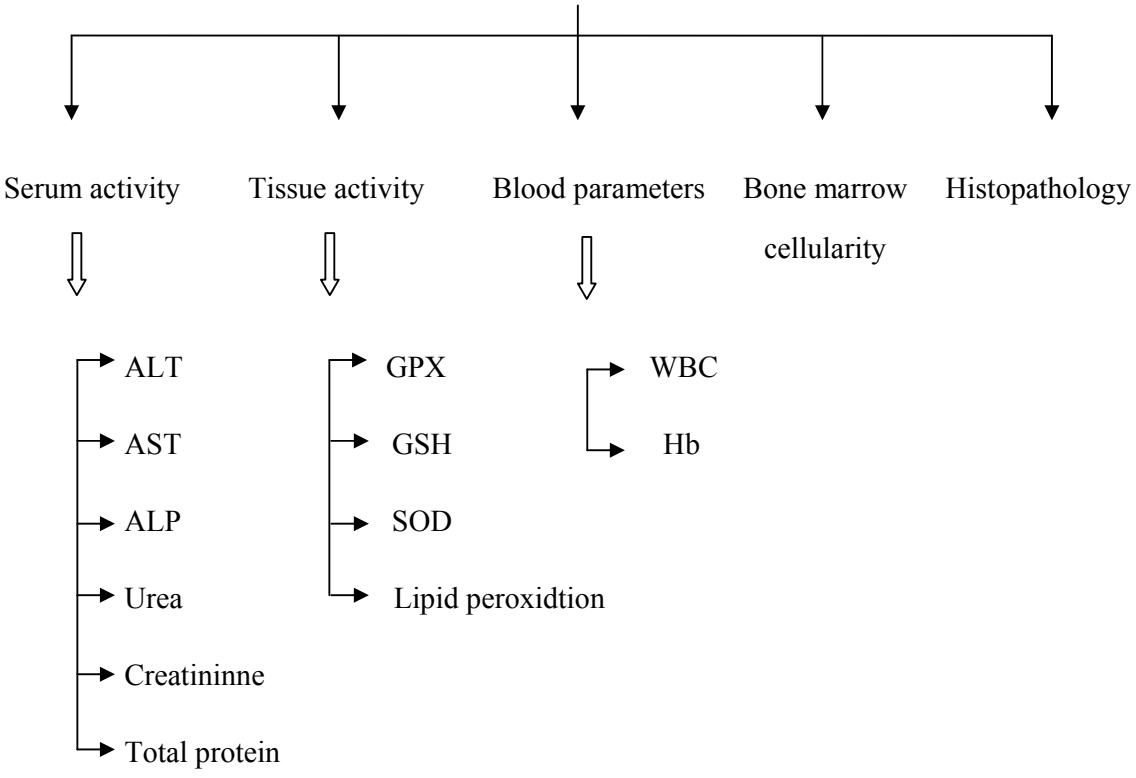
Selection of *Gmelina arborea* stem bark

↓

Preparation of *Gmelina arborea* stem bark 70% methanolic extract using soxhlet extraction apparatus

↓

Evaluation of chemoprotective activity against cyclophosphamide indued activity



# 5. PLANT PROFILE

**Fig 4**: Photograph of *Gmelina arborea* plant (Taken from Amala Ayurvedic garden, Amala Institute of Medical Sciences, Thrissur).

## TAXONOMICAL CLASSIFICATION

**Table: 2 – Taxonomical classification of *G.arborea***

| | |
|---|---|
| Kigdom | Plantae |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Sub class | Asteridae |
| Order | Lamiales |
| Family | Verbenaceae |
| Genus | *Gmelina* |
| Species | *G. arborea* |

## VERNACULAR NAMES

**Table : 3 – Vernacular names of *G.arborea***

| | |
|---|---|
| Assamese | Gomari |
| Bengali | Gamari, Gambar |
| Hindi | Gamhar, Khamara. |
| Kannada | Kooli mara, Kumbuda, Shivane mara |
| Malayalam | Kumbil, Kumbulu |
| Marathi | Shivan, Siwan |
| Sanskrit | Gambhari, Sindhuparni |
| Tamil | Kumla, Kumalamaram |
| Telugu | Gumartek, Gummadi, Summadi |

## BOTANICAL DESCRIPTION

*Gmelina arborea* is a large deciduous tree, which grows on different localities and mainly prefers the moist fertile valleys with 75–450 cm rainfall. It does not thrive on un-drained soils and remains stunted on sandy, dry or poor soils; drought also reduces it to a shrubby form.

The *Gmelina arborea* tree attains height up to 30 m and its wood is pale yellow to cream coloured or plukish-buff when fresh, turning yellowish brown on exposure and is soft to moderately hard, light to moderately heavy, lustrous when fresh, usually straight to irregular or rarely wavy grained and medium course textured.

Leaves are large, tomentose underneath, cordate-ovate, acuminate.

Flowering takes place during February to April when the tree is more or less leafless whereas fruiting starts from May onwards up to June.

The fruit is up to 2.5 cm long, drupe, smooth, dark green, turning yellow when ripe and has a fruity smell.

## DISTRIBUTION

In India, *Gmelina arborea* occurs extensively from the Ravi eastwards in the sub-Himalayan tracts, and it is common throughout Assam and the adjoining areas of

northern West Bengal, Odisha and also in southern Bihar, periodically found in western and southern India and planted elsewhere on a large scale. Gamhar most commonly seen in West Bengal forests (mixed forests). It also occurs naturally in Thailand, Myanmar, Laos, Vietnam, Cambodia and in southern provinces of China.

**MAJOR CHEMICAL CONSTITUENTS**

Lignans such as 4-hydroxysesamin, 6"- bromo–isoarboreol, 4,8-dihydroxysesamin, 1,4-dihydroxysesamin (gummadiol), 2 piperonyl-3-hydroxymethyl-4-(α-hydroxy-3,4-methylenedioxybenzyl)-4-hydroxytetrahydrofuran and the 4-O-glucoside of 4-epigummadiol can be isolated from the heartwood of *Gmelina arborea* (A S R Anjaneyulu *et al*., 1977). The parent compounds are arboreol or gmelanone ( A S R Anjaneyulu *et al*., 1975).

Presence of Umbelliferone 7-apiosylglucoside in the root is reported (P Satyanarayana *et al*., 1985). Other constituents present in plant are β-sitosterol, ceryl alcohol, gmelinol; butyric acid & tartaric acids; apigenin, premnazole, arborone, isoarboreol, cutytyl ferulate, epieudesmin, gmelanore, gmelafuran, gummadiol, saponifiable fraction, apiosyl-skimmin, octacosanol etc.

**TRADITIONAL USES** (Kaswala Rohit *et al*., 2012)

- The bark of *Gmelina arborea* are stomachic, galactogogue, laxative and antihelmenthic, improve appetite, useful in abdominal pains, hallucination, piles, fever, burning sensations, urinary discharge and Tridosha. For the treatment of snake bite and scorpion sting GA is also recommended with other drugs.
- Leaf paste is applied to relieve headache and juice can be used as wash for ulcers. Gamhar leaves, bark skin of Saimali and Apamarga roots are mashed with cow's milk and are given orally to treat hyperacidity. The leaves juice, sugar and milk are recommended in inflammatory condition of urinary bladder and dysuria.
- Flowers are sweet, cooling, acrid, bitter and astringent which is useful in leprosy and blood diseases.
- Fruit is acrid, sour, bitter, sweet, cooling, diueretic, tonic, promote growth of hairs, aphrodisiac, useful in Vata, anaemia, thirst, ulcers, leprosy and vaginal discharge. It is also recommended in raktapitta, excessive thirst, habitual abortion and sexual debility in males. In heart disease of vata imbalance the ripened fruit is valuable.

- The roots are described in the ayurvedic tests as mild laxatives which treats flatulence, increase appetite, lactation and menstrual irregularities. The cold infusion of gamhar, ustra and candana works well with sugar to alleviate the thirst. It is also usefull in piles, burning sensation, fever and 'Tridosha'.

**IMPORTANT PREPARATIONS**

Dasamularishta, Sriparni taila; Draksadi Kvatha, Kasmaryadi panaka, Kasmarya rasayana, Kasmarya taila.

# 6. MATERIALS & METHODS

## 1. PLANT MATERIAL :-

| | |
|---|---|
| Binomial name | : *Gmelina arborea* Roxb. |
| Family | : Verbenaceae |
| Common name | : Gamhar |
| Habit | : Tree |
| Part used | : Stem bark |

***Collection:-*** The stem bark of *Gmelina arborea* Roxb. was collected from Amala Ayurvedic garden, Amala Nagar, Thrissur.

## 2. ANIMALS :-

Male Balb/C mice were purchased from the Small Animal Breeding Station, Agricultural University, Mannuthy, Kerala, India. The animals were maintained under standardized environmental conditions (22-28$^0$C, 60-70% relative humidity, 12hr dark/light cycle) and fed with standard rat feed (Lipton India) and water *ad libitum*. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and followed the guidelines of IAEC(No:- 149/1999/CPCSEA).

## 3. REAGENTS AND CHEMICALS :-

All chemicals used were of analytical quality.

## METHODS

### I. PLANT EXTRACT PREPARATION:-

The stem bark was chipped from the collected stems and was dried in a hot air oven at a temperature of 45$^{o}$C. This was then ground to fine powder and extracted with 70% methanol as the solvent, using a Soxhlet apparatus. The extract was filtered and evaporated to dryness. The dried extract was re-dissolved in distilled water and used for further studies.

### II. ANIMAL GROUPING AND TREATMENT REGIME:-

The animals (with body weights ranging from 25 to 30 gm) were divided into 4 groups of 6 animals each:-

**Group I:** Normal – Untreated.

**Group II:** Control – Cyclophosphamide (20 mg/kg b.wt).

**Group IV:** GALC – *Gmelina arborea* extract low concentration (250 mg/kg b.wt) and Cyclophosphamide (20 mg/kg b.wt).

**Group V:** GAHC - *Gmelina arborea* extract high concentration (500 mg/kg b.wt) and Cyclophosphamide (20 mg/kg b.wt).

The GALC and GAHC groups were orally treated with the extract at doses of 250 mg/kg body weight and 500 mg/kg body weight respectively, for a continuous time period of 20 days, at an administration volume of 0.1 ml for each animal. Within this period, all groups, except normal also received oral administration of cyclophosphamide at a dose of 20 mg/kg body weight, starting from the sixth day upto the fifteenth.

For determining the bone marrow cellularity on the 7$^{th}$ and 14$^{th}$ day, 4 animals each were separately stocked for control, GALC and GAHC groups with the above mentioned treatment regime till their sacrifice. On the initial day of the study, 4 animals separately stocked for the purpose were sacrificed from the normal group.

## III. DETERMINATION OF TOTAL WBC COUNT AND HAEMOGLOBIN LEVEL:-

During the course of the study, the haematological parameters of the animals were tracked on every 5$^{th}$ day. For this, blood was collected from the caudal vein into heparinised tubes and total WBC count and haemoglobin level were checked.

### A. Determination of total count:

**Principle:**

The whole blood was diluted using a diluent which haemolyses red cells, leaving all the nucleated cells intact. The number of white cells in a known volume and known dilution were counted using a counting chamber.

**Procedure:**

0.02 ml of blood was added to 0.38 ml of diluting fluid and mixed well. The diluted blood was charged into a Neubauer counting chamber. After 3-4 min, the total number of white blood cells in the four large corner square chambers was counted.

**Calculation:**

Total WBC = (Number of cells counted x 50) / mm$^3$

### B. Determination of haemoglobin (Hb) content:

Cyanmethaemoglobin method (Kit manufactured by Agappe Diagnostics).

**Principle:**

Haemoglobin was treated with a reagent containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide forms methaemoglobin, which is converted to cyanmethaemoglobin by cyanide. The intensity of colour formed is measured at 546 nm against blank. The optical density is directly proportional to the amount of haemoglobin present in blood.

**Procedure:**

0.02 ml of fresh whole blood was mixed with 5 ml of the cyanmeth reagent. The optical density was measured at 546 nm against blank after 5 min incubation at room

temperature. The OD of standard solution corresponding to 60 mg/dl haemoglobin at 546 nm was also read against reagent blank.

**Calculation:**

Haemoglobin (g/dL) = (OD of treated x 60 x 0.251) / OD of standard

**IV. ESTIMATION OF BODY WEIGHT:**

The body weights of all the animals were measured, starting from the first day, and repeated every fifth day, until sacrifice.

On the 21$^{st}$ day of the study, the animals were sacrificed and the blood and tissue parameters, relative organ weights as well as the bone marrow cellularity were checked.

**V. DETERMINATION OF RELATIVE ORGAN WEIGHTS:-**

After sacrifice, the liver, kidney, spleen, heart and brain were excised and weighed. The percentage weight of each organ, relative to the total body weight was determined.

**VI. DETERMINATION OF BONE MARROW CELLULARITY:-**

Bone marrow cells from both femurs were flushed out with phosphate buffered saline. The number of bone marrow cells were determined using a haemocytometer and expressed as total live cells ($\times 10^6$)/femur.

**VII. EVALUATION OF BIOCHEMICAL PARAMETERS:-**

**1. SERUM PARAMETERS:**

**A. Estimation of Aspartate aminotransferase (AST)/ SGOT:-**

2,4-DNPH (Reitman and Frankel Method)

Kit manufactured by Span diagnostics Ltd. (Cogent) was used for this estimation.

**Principle:**

Aspartate aminotransferase, also known as Glutamate Oxaloacetate Transaminase (GOT) catalyses the transamination of L-aspartate and α keto glutarate to form oxaloacetate and L- glutamate. Oxaloacetate formed is coupled with 2,4- Dinitrophenyl

hydrazine to form hydrazone, a brown coloured complex in alkaline medium which can be colorimetrically measured.

**Reagents:**

Buffered aspartate (pH 7.4); 2,4- DNPH reagent; 4N sodium hydroxide; working pyruvate standard ; solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

**Procedure:**

The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered aspartate was added into all the test tubes. This was followed by the addition of 0.05 ml of serum into the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at $37^{o}C$ for 60 minutes, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 minutes. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was read against purified water in a spectrophotometer at 505 nm within 15 minutes.

The enzyme activity was calculated as:-

AST (GOT) activity in IU/L) =

[(Absorbance of test - Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard

**B. Estimation of Alanine Aminotransferase (ALT)/ SGPT:-**

2,4-DNPH (Reitman and Frankel Method)

Kit manufactured by Span diagnostics Ltd.(Cogent) was used for this estimation.

**Principle:**

Alanine aminotransferase also known as Glutathione Peroxidase (GPT) catalyses the transamination of L-alanine and α keto glutarate to form pyruvate and L- Glutamate. Pyruvate so formed is coupled with 2,4 – Dinitrophenyl hydrazine to form a corresponding hydrazone, a brown coloured complex in alkaline medium which can be measured colorimetrically.

**Reagents:**

Buffered alanine (pH 7.4), 2,4–DNPH, 4N sodium hydroxide, working pyruvate standard, solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

**Procedure:**

The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered alanine was added into all the test tubes. This was followed by the addition of 0.05 ml of serum into the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at $37^{o}$C for 60 minutes, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 minutes. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was read against purified water in a spectrophotometer at 505 nm within 15 minutes.

The enzyme activity was calculated as:-

ALT (GPT) activity in IU/L) =

[(Absorbance of test - Absorbance of control) / (Absorbance of standard - Absorbance of blank)] x concentration of the standard

**C. Estimation of Alkaline phosphatase:-**

Kind & King's method

Kit manufactured by Span diagnostics Ltd.(Cogent) was used for this estimation.

**Principle:**

Alkaline phoshatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidising agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured spectrometrically. The color intensity is proportional to the enzyme activity.

**Reagents:**

Buffered substrate

Chromogen Reagent

Phenol Standard, 10 mg%

**Procedure:**

The working solution was prepared by reconstituting one vial of buffered substrate with 2.2 ml of water. 0.5ml of working buffered substrate and 1.5 ml of purified water was dispensed to blank, standard, control and test. Mixed well and incubated at $37^0$C for 3 minutes. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively. Mixed well and incubated for 15 minutes at $37^0$C. Thereafter, 1 ml of chromogen reagent was added to all the test tubes. Then, added 0.05 ml of serum to control. Mixed well after addition of each reagent and the O.D of blank, standard, control and test were read against purified water at 510nm.

**Calculation:**

Serum alkaline phosphatase activity in KA units =

[(O.D. Test-O.D. Control) / (O.D. Standard- O.D. Blank)] x 10

**D. Estimation of Creatinine:-**

Jaffe's Kinetic method

Kit manufactured by Asritha Diatech India (Euro) was used for this estimation.

**Principle:**

Creatinine is the catabolic product of creatinine phosphate which is used by the skeletal muscle. The daily production depends upon the muscular mass and it is excreted out of the body through kidney. Creatinine present in the sample generates a coloured complex upon reacting with sodium picrate.

creatinine + sodium picrate → creatinine-picrate complex

**Reagents:**

R1- Picric acid reagent

R2- Alkaline buffer

Creatinine standard - 2 mg/ dl.

**Procedure:**

Working reagent (WR) was prepared by mixing equal volumes of R1 and R2. The reaction systems consisted of standard and test solutions. 1 ml of WR was dispensed to all the tubes, followed by the addition of 0.05 ml of creatinine standard and 0.05 ml of serum into the standard and test sample tubes respectively. Mixed well and read the absorbance of standard and test against distilled water at 520 nm after 30 sec ($A_0$) and 90 sec ( $A_1$)

ΔA for standard (S) and Test (T) was determined as:-

$\Delta A_s = A_{S1} - A_{S0}$

$\Delta A_T = A_{TI} - A_{TO}$

Serum creatinine (mg/dl) = $(\Delta A_T/\Delta A_s) \times 2$

**E. Estimation of Serum Urea:-**

Estimation was done by Berthelot Enzymatic method for the determination of urea in serum.

**Principle:**

Urea is converted quantitatively by urease into ammonia and carbon di oxide. The ammonium ions react with hypochlorite and salicylate to give a green coloured complex. The colour is enhanced by sodium nitroprusside. The intensity of color s directly related to urea concentration and is measuresd photometrically at 578 nm.

**Reagents:**

R1- urease and sodium salicylate ; R 2- alkaline hypochlorite solution ; standard 40 mg/dl

**Procedure:**

Working reagent was prepared by the addition of 25 ml of distilled water to R1. From this 1ml of solution was pip petted out into all the test tubes. 0.01 ml of distilled water was added into the blank test tube followed by 0.01 ml of standard into the standard tube and 0.01 ml of serum into the test tubes. Mixed well and incubated for 5 minutes at 37□c followed by addition of 1 ml of R2 into all the test tubes. Mixed well and incubated for 5 minutes at 37□c. At the end of incubation absorbance of standard and test was measured against blank at 578nm.

Urea mg/dl = $A_t / A_s \times 40$

**F. Estimation of Serum Total Protein:-**

Biuret method

Kit manufactured by Asritha Diatech India (Euro) was used for this estimation.

**Principle:**

Protein + $Cu^{++}$ → Blue- violet coloured complex

The intensity of the colour formed is directly proportional to the amount of total proteins present in the sample.

**Reagents:**

Biuret reagent, Protein standard (6g/dl)

**Procedure:**

1 ml Biuret reagent was added into all the test tubes followed by 0.01 ml of total protein standard into the standard tube and 0.01 ml of serum into the test groups. Mixed well and incubated at 37˚c for 5 minutes.Measured the absorbance of standard and test sample against blank at 555nm within 60 minutes.

**Calculation:**

Total protein (g/dl) = (O.D. test / O.D. std.) x 6 g/dl

**2. TISSUE ANTI-OXIDANT PARAMETERS:-**

**Preparation of Tissue Homogenates (Liver & Kidney):-**

On 21$^{st}$ day, animals were sacrificed. Liver and kidney were excised and rinsed thoroughly in ice cold saline to remove the blood. They were gently blotted between the folds of thoroughly in ice-cold saline to remove any blood traces. They were then gently blotted between the folds of a filter paper and weighed in an analytical balance. 25% of homogenate was prepared in 0.05M phosphate buffer (pH 7) using a polytron homogenizer at 4˚C. A part of this homogenate was used for the determination of total protein & lipid peroxidation (LPO). Rest of the homogenate was centrifuged at 10,000 rpm for 30 minutes in a cooling centrifuge for removing the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for the estimation of superoxide dismutase (SOD), total protein, glutathione peroxidase (GPx) and glutathione (GSH).

**A. Estimation of Tissue Superoxide Dismutase (SOD) Activity:-**

**Principle:**

The assay is based on the ability of enzyme to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduced riboflavin with oxygen.

**Reagents:**

M/15 Phosphate buffer, pH 7.8, EDTA (0.1M) containing 1.5 mg of sodium cyanide per 100ml, riboflavin (0.12mM), stored cold in a dark bottle and NBT (1.5mM) stored cold.

**Procedure:**

For each sample to be assayed, the amount of enzyme added to the medium was kept below 1 unit of enzyme activity. Incubation medium contained 0.1ml of tissue sample, 0.2ml EDTA/NACN, 0.1ml NBT, and 0.05ml riboflavin and phosphate buffer to give a total volume of 3ml. The tube without animal sample was kept as control. Riboflavin was added after the tubes were brought to room temperature. Then the tubes were placed in a bright box where they received uniform illumination for 15 minutes. The optical densities were measured at 560nm. One unit of enzyme activity is defined as amount of enzyme giving 50% inhibition of the reduction of NBT and expressed as units/mg. protein.

**B. Estimation of Glutathione (GSH):-**

**Principle:**

GSH is measured by its reaction with DTNB to give a yellow colored complex with maximum absorption at 412nm.

**Reagents:**

25% TCA, 5% TCA, 0.2M GSH buffer (pH 8.0), 0.6 mM 5, 5'-dithiobis 2-nitrobenzoic acid (DTNB).

**Procedure:**

To 0.1 ml of homogenate 125µl of 25% TCA was added to precipitate proteins. The tubes were cooled by keeping on ice bags for 5 minutes and the mixture was further diluted with 0.6 ml. of 5% TCA; centrifuged for 10 minutes and 0.1 ml of resultant supernatant was taken for GSH estimation. The volume of the aliquot was made up to 1 ml with 233µl of 0.2M phosphate buffer (pH 8.0) and 667 µl of freshly prepared 0.6mM

DTNB The intensity of yellow colour formed by DTNB addition was read at 412 nm with distilled water as reference. Standard curve of GSH was prepared using concentrations varying from 5-100 nmol in 5% TCA for each assay. Value was expressed as nmol / mg protein.

**C. Estimation of Glutathione Peroxidase (GPx) Activity:**

**Principle:**

Glutathione peroxidase degrades $H_2O_2$ in presence of glutathione (GSH) thereby depleting it. GSH remaining is measured using DTNB, which gives a coloured complex.

**Reagents:**

1M Phosphate buffer (pH 7.0), 0.2 mM GSH , 25mM Sodium azide, 1.2 mM $H_2O_2$, 0.4 M $Na_2HPO_4$ and -1mM 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB).

**Procedure:**

The reaction mixture containing 0.1 ml each of tissue sample, GSH, $NaN_3$ and $H_2O_2$ was made up to a total volume of 2.5 ml using phosphate buffer and was incubated at 37°C for 6 minutes. After addition of 2 ml. of 1.67 % $HPO_3$, this mixture was centrifuged at 800×g for 15 minutes. 667µl of the supernatant was added to a mixture of 1ml of $Na_2HPO_4$ and DTNB. After 10 minutes of incubation at 37°C, the absorbance of the reaction mixture was measured at 412 nm, with distilled water as reference. The enzyme activity was expressed as units/mg protein.

**D. Estimation of Tissue Lipid Peroxidation**

**Principle:**

Malondialdehyde (MDA) produced during peroxidation can react with thiobarbituric acid (TBA) reagent to form a pink coloured product which has an absorption maximum at 532nm. The assay is calibrated with 1,1,3,3,

tetramethoxypropane, which on hydrolysis produces malondialdehyde. The results are expressed in terms of the amount of malondialdehyde produced during the reaction.

**Reagents:**

150 mM KCl, 0.3 mM ascorbic acid, 0.8mM ferrous ammonium sulphate, 0.2 M tris buffer (pH 7.0), 0.8% TBA, 8.1% SDS and 20% acetic acid (pH 3.5).

**Procedure:**

0.1ml. of the tissue sample (25%) in Tris buffer (pH 7.0) was added to a reaction mixture containing KCl (0.1 ml.), ascorbic acid (0.1ml.), ferrous ammonium sulphate (0.1 ml) and tris buffer (0.1 ml.) mixed into a final volume of 0.5 ml. The reaction mixture was incubated for 1hr at 37°C. The amount of MDA formed was expressed as nmol/mg. protein.

**VII. HISTOPATHOLOGY:-**

A portion of small intestine belonging to one animal in each group was preserved in 10% formaldehyde solution for histopathological studies. Hematoxylin and eosin were used for staining; later, the histopathological slides were photographed.

**VIII. STATISTICAL ANALYSIS:-**

The experimental results are presented as Mean ± SD for 5 animals in each group. Statistical evaluation of the data was done by one way ANOVA followed by Dunnet's t-test. Results were considered statistically significant when $p<0.05$.

# 7. RESULTS

## 1. PLANT EXTRACT PREPARATION:-

The stem bark was chipped from the collected stems and was dried in a hot air oven at a temperature of 45$^{o}$C. This was then ground to fine powder and extracted with 70% methanol as the solvent, using a Soxhlet apparatus. The extract was filtered and evaporated to dryness. The dried extract was re-dissolved in distilled water and used for further studies.

**Table: 4 -** Characteristics of *Gmelina arborea* stem bark extract.

| Name of Extract | Colour | Consistency | Yield (%W/W) |
|---|---|---|---|
| 70% Methanolic Extract | Black | Sticky | 9.5 |

## 2. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON TOTAL WBC COUNT:-

The results revealed that the control animals treated with cyclophosphamide alone showed lower levels of WBC count, when compared to the normal reference group. On treatment with GA extract, the WBC levels of GALC and GAHC were replenished to its normal states (Table 5 & Fig 5).

**Table: 5 -** Effect of *Gmelina arborea* on Total WBC Count

| | NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|---|
| 0th DAY | 13.01±0.578 | 13.16±0.684 | 13.17±0.726 | 12.8±0.654 |
| 5th DAY | 12.933±0.416 | 11.033±0.55 | 12.966±0.208 | 12.183±0.325 |
| 10th DAY | 12.833±0.321 | 8.466±0.901 | 9.916±0.579 | 10.8±0.4 |
| 15th DAY | 12.166±0.553 | 6.483±0.86 | 07.76±0.375 | 9.7±0.4 |
| 20th DAY | 12.033±0.404 | 5.8±0.556 | 6.133±0.45 | 10.346±0.313 |

Values are expressed as mean ± SD for 6 animals.

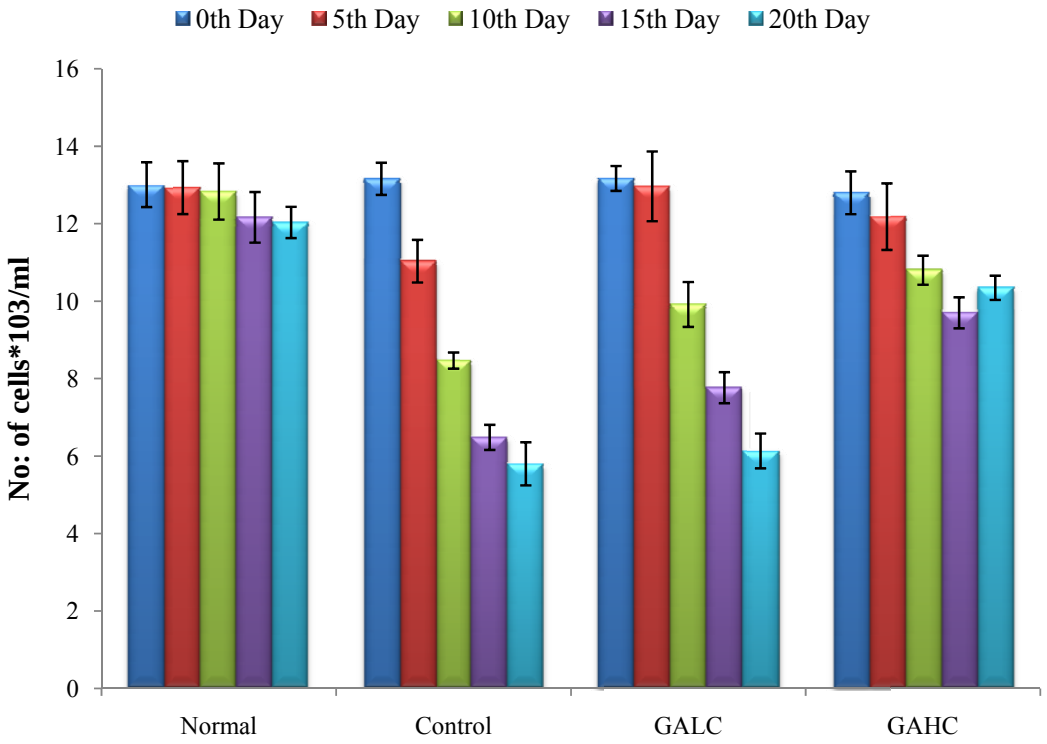



**Fig :5 -** Effect of *Gmelina arborea* on Total WBC Count **(**Values are expressed as mean ± SD for 6 animals).

### 3. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON HAEMOGLOBIN LEVEL:-

The Cyclophosphamide treated group did not show any significant difference in the haemoglobin level up to the 20$^{th}$ day, when compared to the normal group. The plant extract treated groups also did not show any notable changes in the level of haemoglobin (Table 6 & Fig 6).

**Table: 6 -** Effect of *Gmelina arborea* on Haemoglobin level

| | NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|---|
| **0$^{th}$ DAY** | 12.972±0.931 | 12.866±0.365 | 12.725±121.563 | 13.377±0.447 |
| **5$^{th}$ DAY** | 12.566±0.424 | 12.588±0.365 | 12.563±0.440 | 13.077±0.345 |
| **10$^{th}$ DAY** | 13.059±0.543 | 12.948±0.287 | 12.049±0.201 | 12.776±0.842 |
| **15$^{th}$ DAY** | 12.461±0.6119 | 12.810±0.832 | 13.029±0.685 | 12.478±0.518 |
| **20$^{th}$ DAY** | 12.910±0.218 | 12.578±0.268 | 13.035±0.717 | 13.035±0.503 |

Values are expressed as mean ± SD for 6 animals

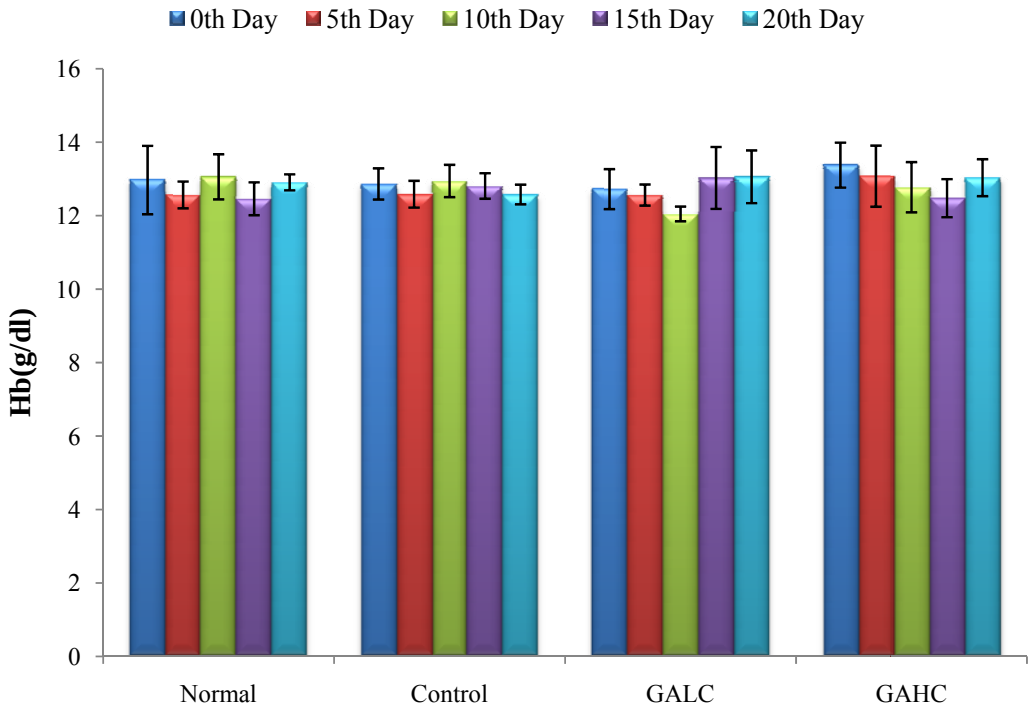



**Fig : 6 -** Effect of *Gmelina arborea* on Haemoglobin level **(**Values are expressed as mean ± SD for 6 animals).

## 4. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON BODY WEIGHT:

Results revealed that, the administration of *Gmelina arborea* extract does not produce any significant changes in the body weight of the animals during the period of study. The cyclophosphamide alone treated control group has showed a slight decrease in the body weight of the animals (Table 7 & Fig 7).

**Table : 7 -** Effect of *Gmelina arborea* on body weight

| | $0^{TH}$ DAY | $5^{TH}$ DAY | $10^{TH}$ DAY | $15^{TH}$ DAY | $20^{TH}$ DAY |
|---|---|---|---|---|---|
| **NORMAL** | 30.3±1.189 | 28.44±2.018 | 26.52±0.929 | 28.28±1.136 | 29.22±1.657 |
| **CONTROL** | 31.68±1.294 | 29.11±1.292 | 27.84±1.384 | 28.87±1.634 | 28.52±1.395 |
| **GALC** | 26.38±1.031 | 27.57±1.557 | 26.92±1.939 | 27.06±1.436 | 27.06±1.436 |
| **GAHC** | 25.8±1.644 | 25.48±0.897 | 26.08±1.109 | 24.82±1.035 | 24.08±0.769 |

Values are expressed as mean ± SD for 6 animals.

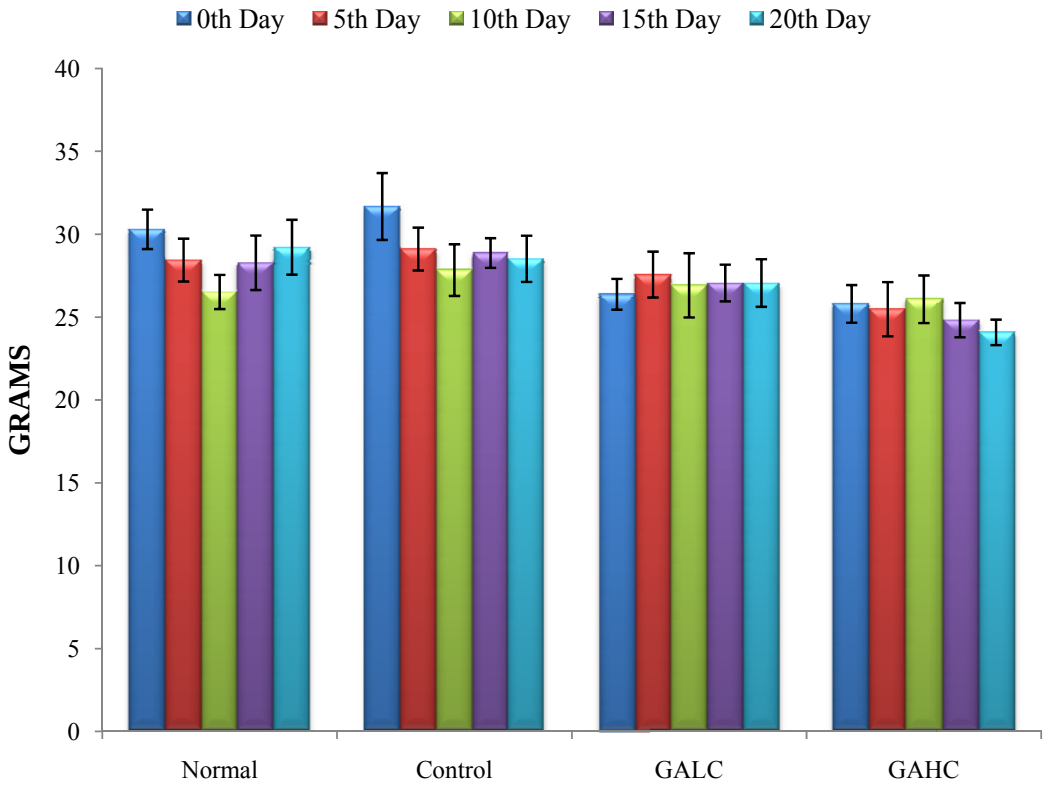



**Fig: 7 -** Effect of *Gmelina arborea* on body weight (Values are expressed as mean ± SD for 6 animals).

## 5. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON RELATIVE ORGAN WEIGHTS:

The weight of heart, kidney and brain of all animals of all group were found to be in similar range to that of the normal group. Whereas, the spleen weight of control and GALC groups were lowered when compared to the normal. While that of the GAHC group has improved the relative organ weight when compared to the control (Table 8 and Fig 8).

**Table: 8** - Effect of *Gmelina arborea* on relative organ weight

| | HEART | KIDNEY | LIVER | SPLEEN | BRAIN |
|---|---|---|---|---|---|
| **NORMAL** | 0.499±0.095 | 1.323±0.061 | 5.576±0.601 | 1.221±0.18 | 1.215±0.153 |
| **CONTROL** | 0.538±0.059 | 1.315±0.189 | 5.052±0.068 | 0.409±0.101 | 1.214±0.159 |
| **GALC** | 0.517±0.017 | 1.333±0.1 | 5.482±0.638 | 0.467±0.08 | 1.251±0.132 |
| **GAHC** | 0.514±0.034 | 1.432±0.115 | 5.056±0.399 | 0.831±0.12 | 1.559±0.075 |

Values are expressed as mean ± SD for 6 animals.

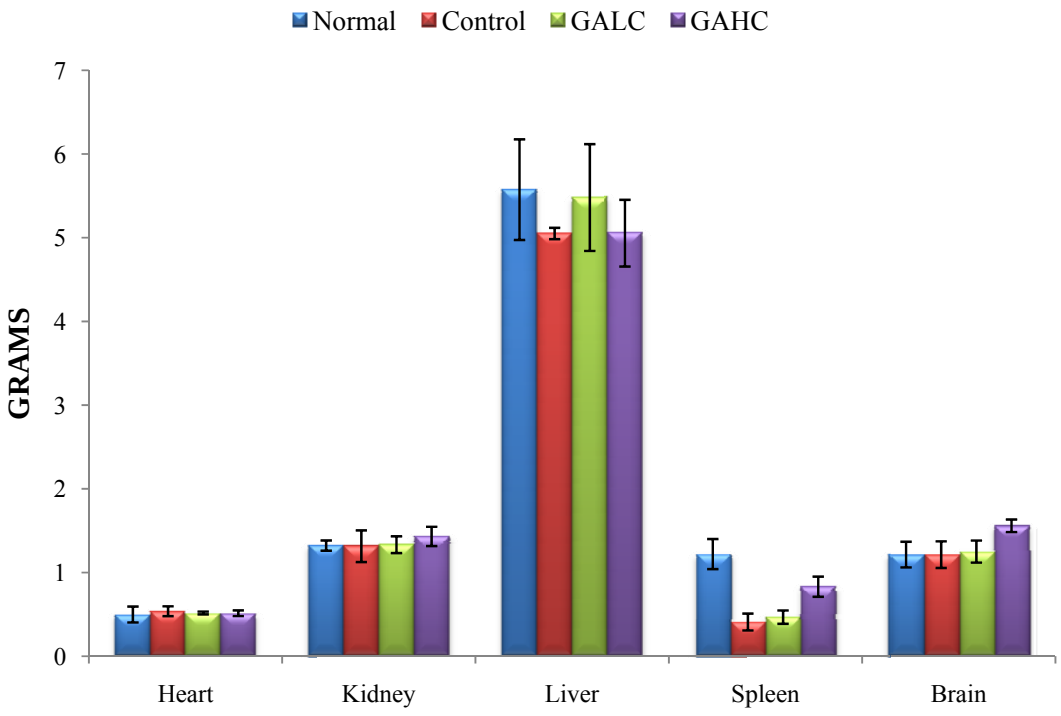



**Fig : 8** - Effect of *Gmelina arborea* on relative organ weight (Values are expressed as mean ± SD for 6 animals).

## 6. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON BONE MARROW CELLULARITY (BMC):-

The bone marrow cellularity was found to be lower in the cyclophosphamide treated group compared to normal, GALC and GAHC groups (Table 9 & Fig 9). The treatment with low and high doses of GA replenished the BMC to the normal range.

**Tab: 9** - Effect of *Gmelina arborea* on Bone Marrow Cellularity

| | NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|---|
| **INITIAL** | 1322±72 | - | - | - |
| **7TH DAY** | - | 1089.22±74 | 918.75±89 | 834.5±51 |
| **14TH DAY** | - | 1234.55±99 | 1107.32±71 | 1097.41±67 |
| **21ST DAY** | 1299.87±63 | 1216.42±38 | 1154.86±33 | 1143.98±56 |

Values are expressed as mean ± SD for 6 animals.

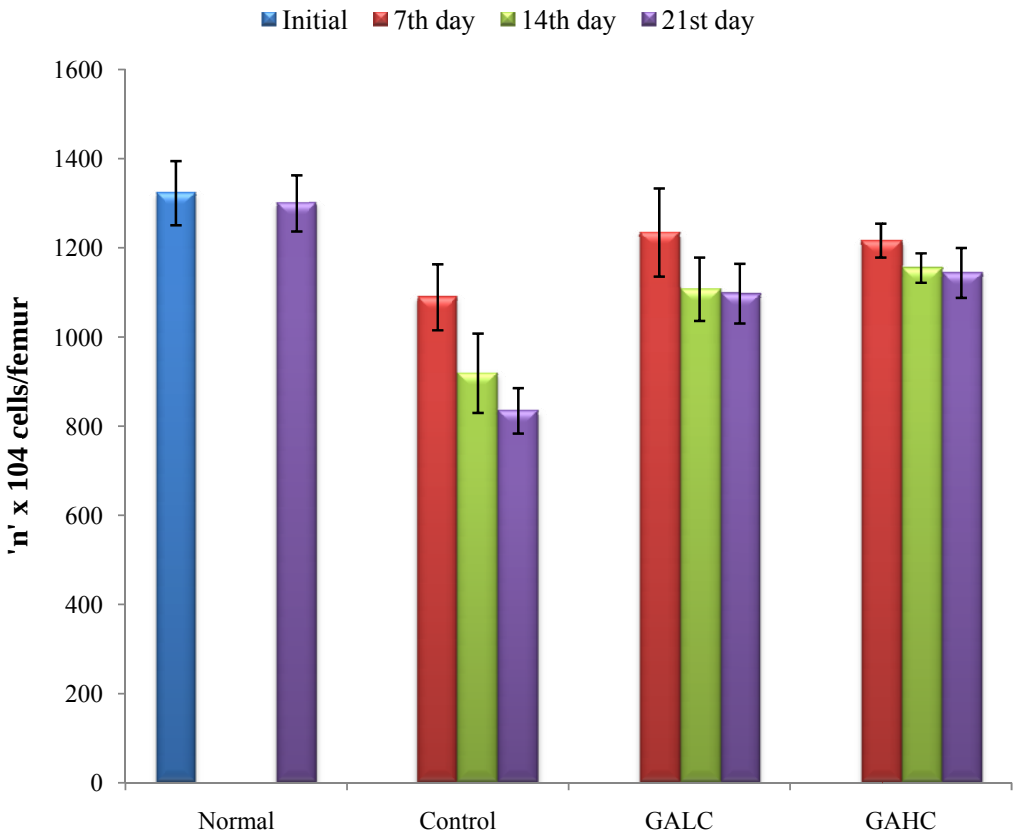



**Fig: 9** - Effect of *Gmelina arborea* on Bone Marrow Cellularity (Values are expressed as mean ± SD for 6 animals).

## 7. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON SERUM PARAMETERS:

### A) ESTIMATION OF ASPARTATE AMINOTRANSFERASE (AST/ SGOT):-

The results revealed that the control animals treated with cyclophosphamide alone showed higher levels of the serum marker GOT (129.165 ± 8.367U/L), compared to the normal reference group (108.234 ± 6.186 U/L). On treatment with GA extract, there was a decrease in the level of SGOT with values recorded as 121.386±6.382 U/L and 115.32±7.82 U/L respectively for GALC and GAHC groups (Table10 & Fig10).

**Table: 10 -** Effect of *Gmelina arborea* on Aspartate aminotransferase (IU/L)

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 108.234±6.186 | 129.165±8.367[a] | 121.386±6.382 | 115.32±7.82[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

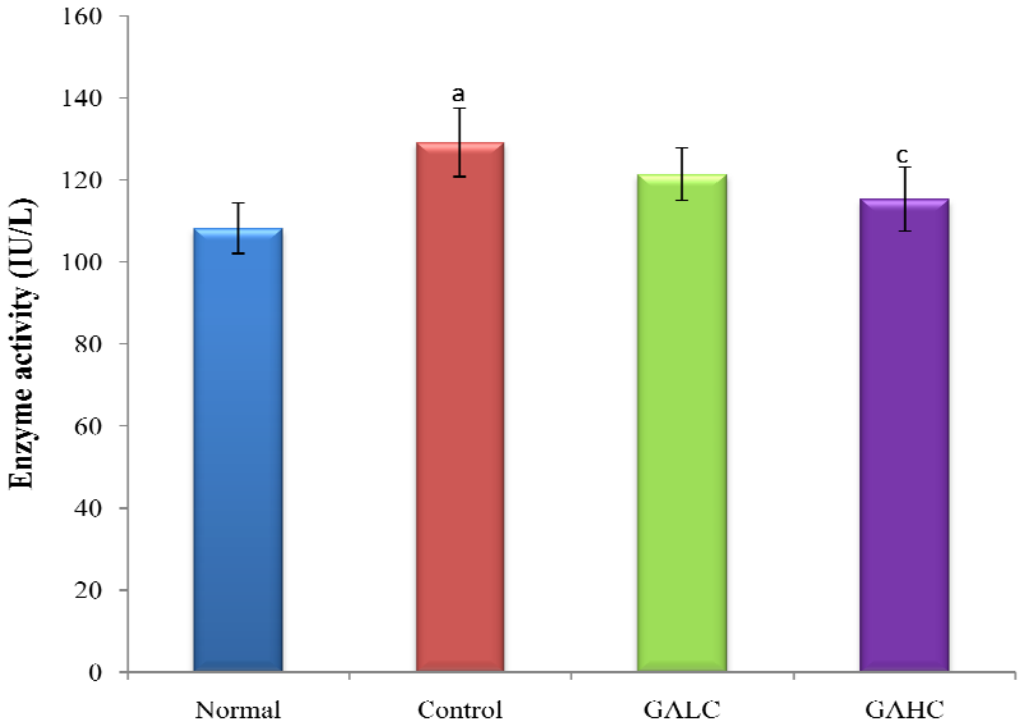



**Fig: 10 -** Effect of *Gmelina arborea* on aspartate aminotransferase (AST/ SGOT) (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**B) ESTIMATION OF ALANINE AMINOTRANSFERASE (ALT)/ SGPT:-**

This liver specific serum marker GPT showed a steep increase in the control group **(**82.2±8.833U/L), while the normal group SGPT level was 42.975± 3.465 U/L. This was indicative of liver damage by cyclophosphamide administration in the control animals without any drug supplementation. A lowering of SGPT level was found in the GA treated low and high dose groups where the readings were 72.384±7.212 U/L and 55.54±5.638 U/L respectively (Table 11 & Fig 11).

**Table: 11 -** Effect of *Gmelina arborea* on Alanine Aminotransferase (IU/L)

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 42.975± 3.465 | 82.2±8.833[a] | 72.384±7.212[d] | 55.54±5.638[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

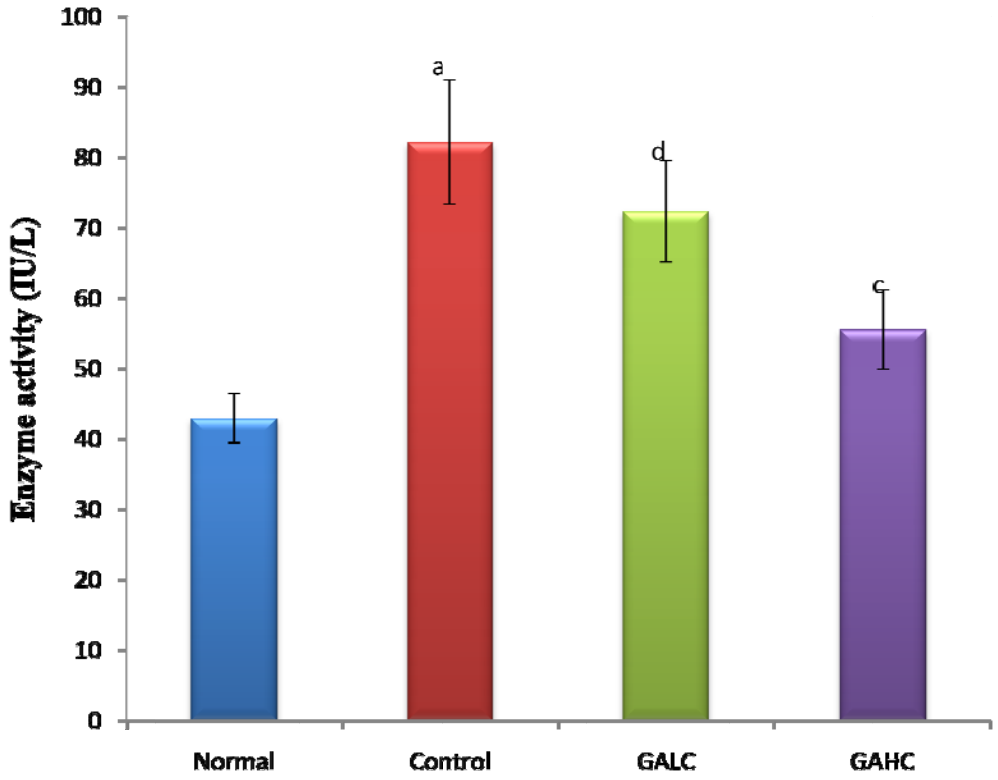



**Fig: 11 -** Effect of *Gmelina arborea* on Alanine Aminotransferase (ALT)/ SGPT**)** (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

## C) ESTIMATION OF ALKALINE PHOSPHATASE (ALP) :-

The ALP concentration was found to be higher in the untreated control group (84.963±6.629 U/L). The GALC and GAHC groups produced mean ALP activities of 76.297 ± 5.729 U/L and 65.83 ± 5.827 U/L respectively, showing moderate restoration towards normal value (60.217±4.739 U/L) (Table 12 & Fig 12).

**Table: 12 -** Effect Of *Gmelina arborea* on Alkaline Phosphatase ( IU/L)

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 60.217 ± 4.739 | 84.963 ± 6.629[a] | 76.297 ± 5.729[d] | 65.83 ± 5.827[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

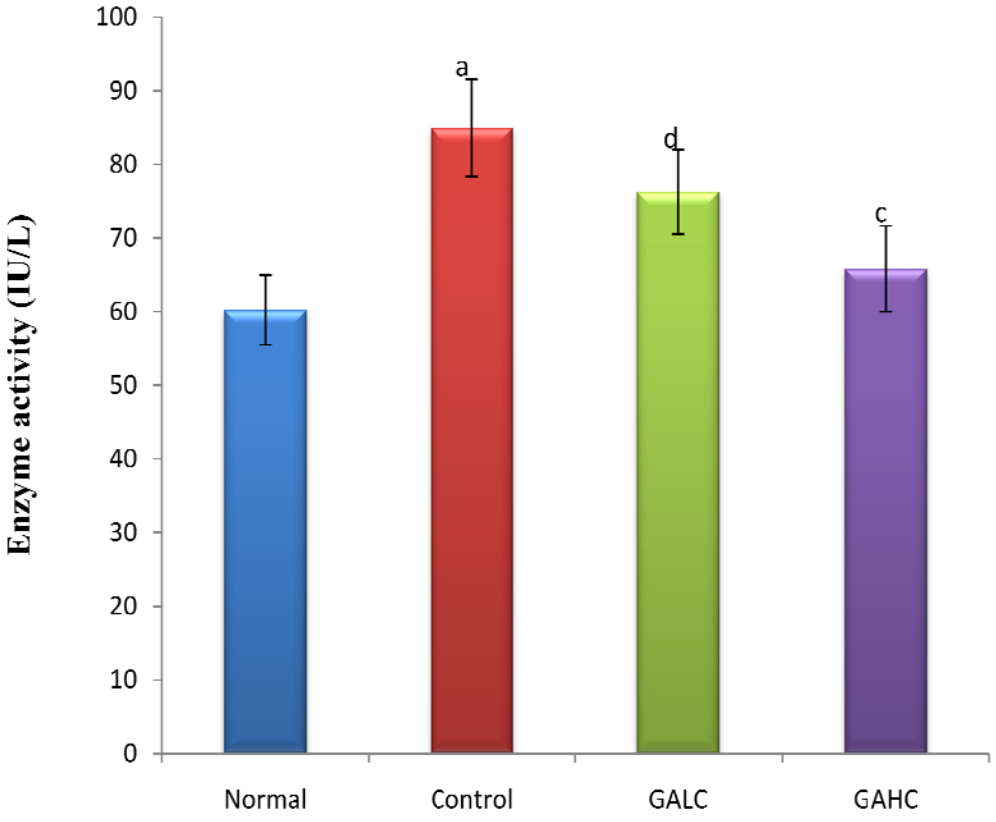



**Fig: 12 -** Effect Of *Gmelina arborea* on Alkaline Phosphatase (ALP) (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**D) ESTIMATION OF SERUM CREATININE:-**

The creatinine concentration was found to be higher in the cyclophosphamide alone treated control group (1.25± 0.061 mg/dl) compared to the normal (0.666 ± 0.028 mg/dl). The treatment with low and high doses of GA showed a slight decrease in the creatinine range, i.e; 1.083 ± 0.026 mg/dl and 1 ±0.031 mg/dl respectively (Table 13 & Fig 13).

**Table : 13** - Effect of *Gmelina arborea* on serum Creatinine (mg/dl)

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 0.666 ± 0.028 | 1.25± 0.061[a] | 1.083 ± 0.026[c] | 1 ± 0.031[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

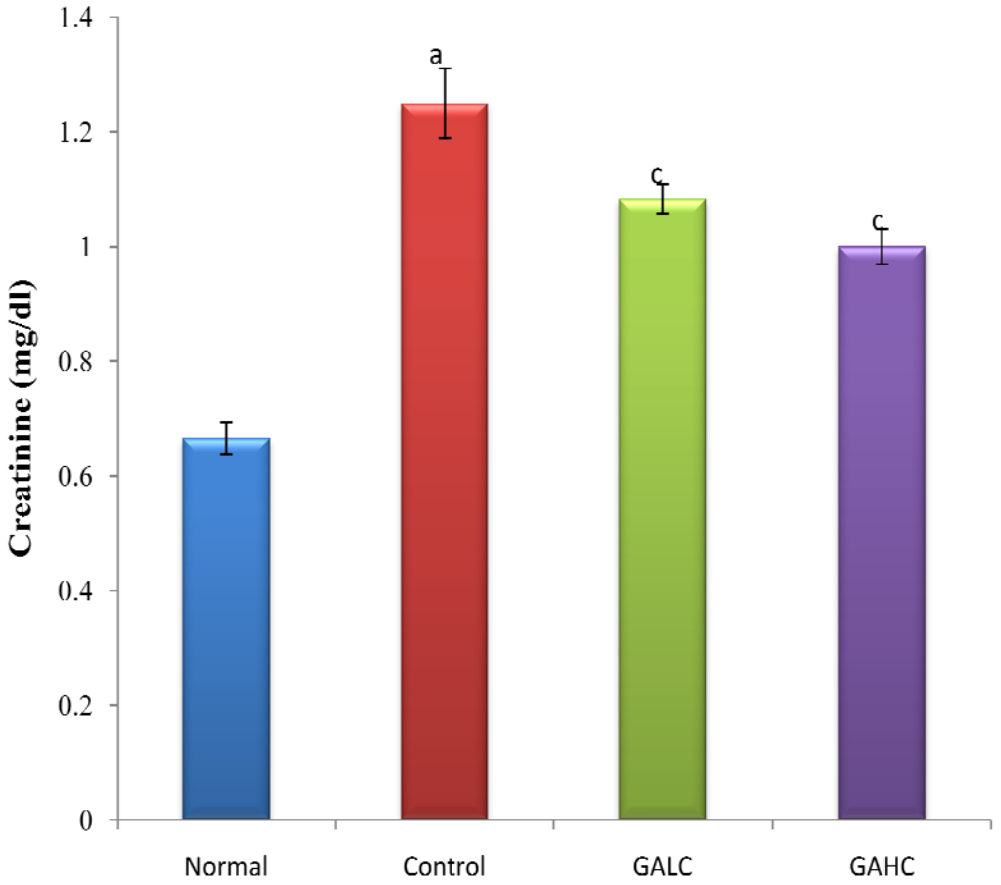



**Fig : 13** - Effect of *Gmelina arborea* on Creatinine (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**E) ESTIMATION OF SERUM UREA:-**

The urea concentration was found to be higher in the control group (27.335 ± 3.205 mg/dl) compared to the normal range (17.871 ± 2.268 mg/dl). The GALC and GAHC groups produced mean values of 20.087 ± 3.432 mg/dl and 18.457 ± 3.293 mg/dl indicating gradual restoration to normal values (Table 14 & Fig 14).

**Table :14** - Effect of *Gmelina arborea* on Serum Urea (mg/dl)

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 17.871 ± 2.268 | 27.335 ± 3.205[a] | 20.087±3.432[d] | 18.457±3.293[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

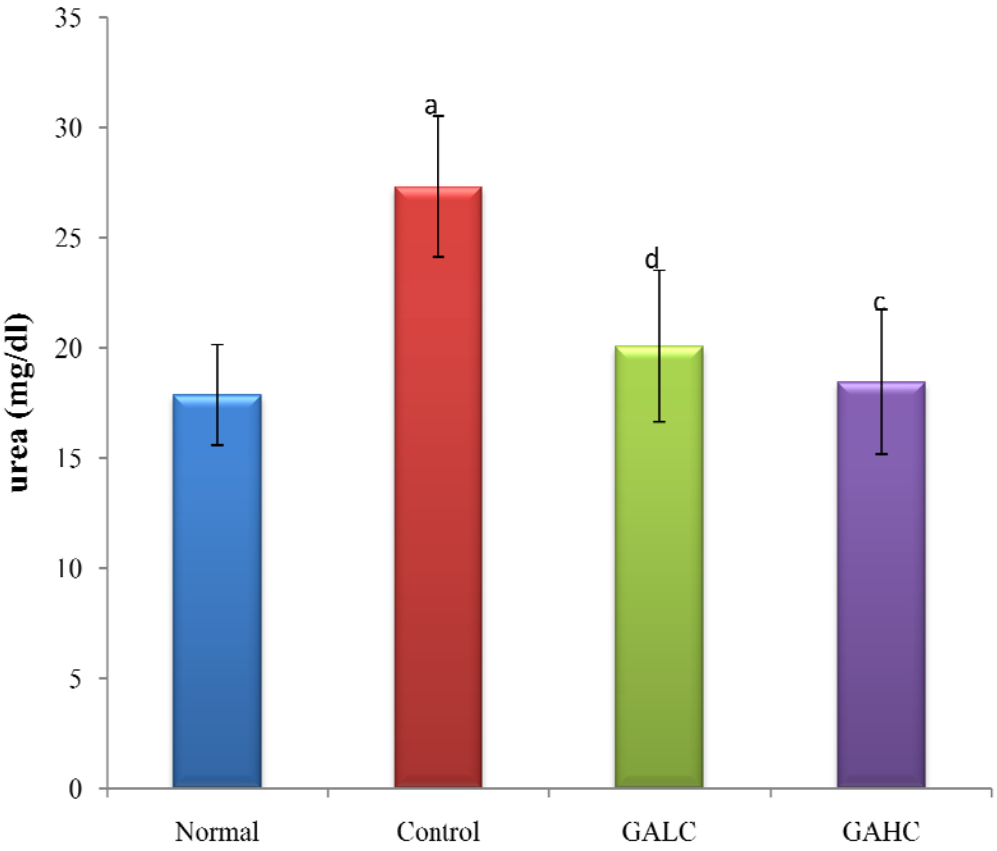



**Fig : 14** - Effect of *Gmelina arborea* on serum Urea ( Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**Table: 15 -**Effect of *Gmelina arborea* on all serum parameters

| SERUM PARAMETERS | NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|---|
| **AST/ SGOT** ( IU/L) | 108.234±6.186 | 129.165±8.367[a] | 121.386±6.382 | 115.32±7.82[c] |
| **ALT/ SGPT** ( IU/L) | 42.975± 3.465 | 82.2±8.833[a] | 72.384±7.212[d] | 55.54±5.638[c] |
| **ALP** (KA) | 60.217 ± 4.739 | 84.963 ± 6.629[a] | 76.297 ± 5.729[d] | 65.83 ± 5.827[c] |
| **CREATININE** (mg/dl) | 0.666 ± 0.028 | 1.25± 0.061[a] | 1.083 ± 0.026[c] | 1 ± 0.031[c] |
| **UREA** (mg/dl) | 17.871 ± 2.268 | 27.335 ± 3.205[a] | 20.087±3.432[d] | 18.457±3.293[c] |

Values are expressed as mean ± SD for 6 animals; a :- $p<0.01$ and b :- $p<0.05$ compared to normal ; c:- $p<0.01$ and d:- $p<0.05$ compared to control.

## 8. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON TISSUE ANTI-OXIDANT PARAMETERS:-

### A.1) ESTIMATION OF LIVER SUPEROXIDE DISMUTASE (SOD) ACTIVITY:

SOD level was found to be lower in the control group (0.382± 0.039 U/mg). It was markedly replenished to normal state (0.679 ± 0.078 U/mg) by GALC (0.509± 0.051 U/mg) and GAHC (0.58 ±0.046 U/mg) drug regimen respectively (Table 16 & Fig 15).

**Table : 16** - Effect of *Gmelina arborea* on liver Superoxide Dismutase Activity (SOD) (Enzyme activity in U/mg protein).

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 0.679 ± 0.078 | 0.382± 0.039[a] | 0.509±0.051[c] | 0.58±0.046[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control

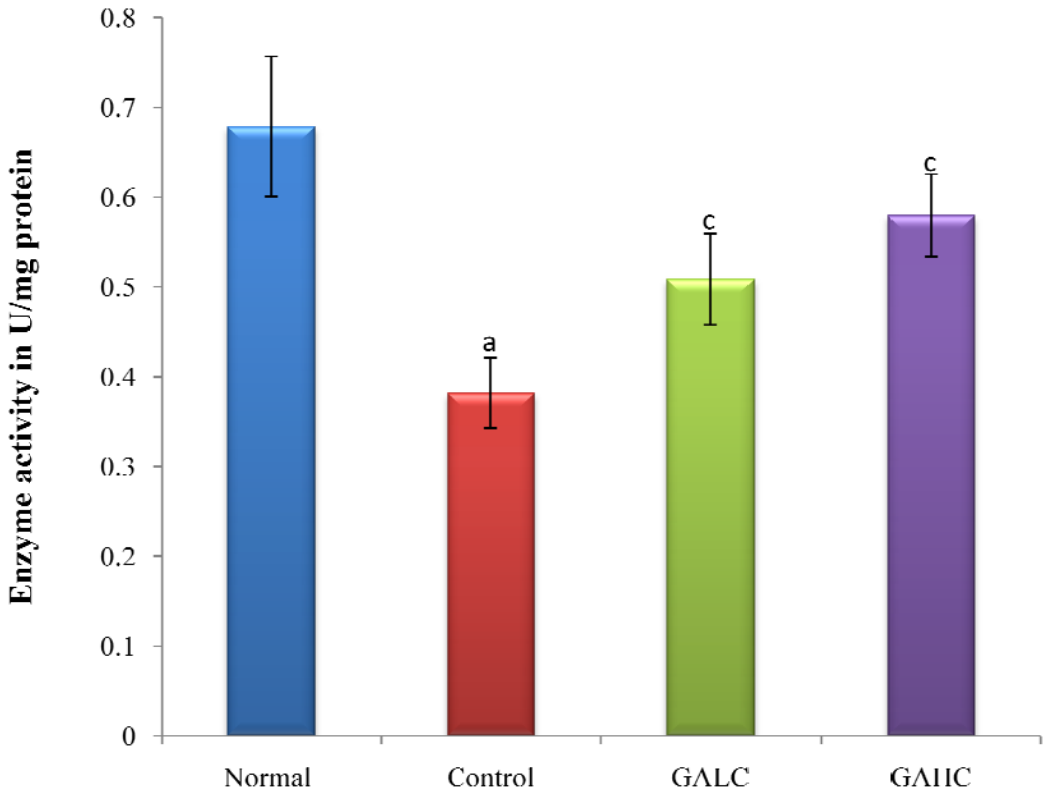



**Fig :15** - Effect of *Gmelina arborea* on liver Superoxide Dismutase activity (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**A.2) ESTIMATION OF KIDNEY SUPEROXIDE DISMUTASE (SOD) :-**

SOD level was found to be lower in the control group (0.577 ± 0.087 U/mg). It was replenished to normal range (0.805 ± 0.093 U/mg) by the high and low doses of GAHC (0.72± 0.096 U/mg) and GALC (0.68± 0.01 U/mg) drug regimen respectively (Table 17 & Fig 16).

**Table : 17 -** Effect of *Gmelina arborea* on kidney superoxide dismutase (SOD) (Enzyme activity in U/mg protein).

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 0.805 ± 0.093 | 0.577 ± 0.087[a] | 0.68 ±.01 | 0.72 ± 0.096[d] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control

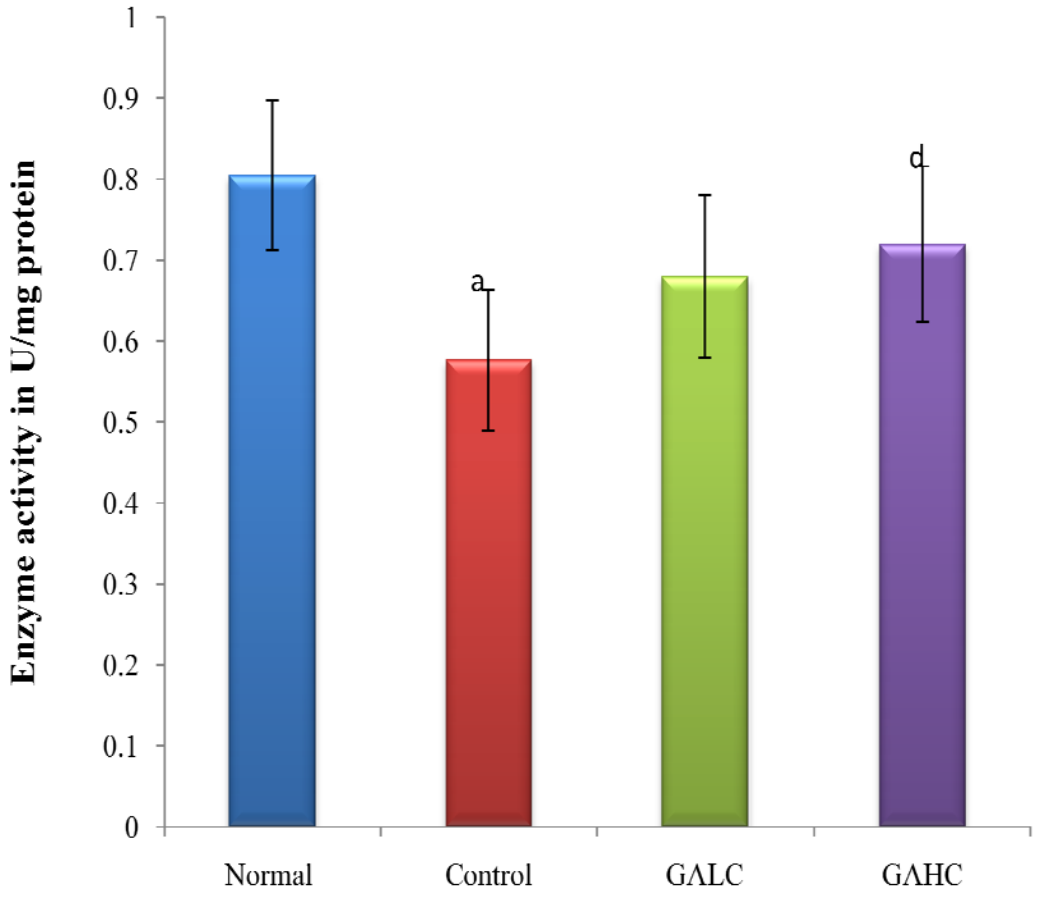



**Fig:16 -** Effect of *Gmelina arborea* on kidney superoxide dismutase (SOD) (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**B.1) ESTIMATION OF LIVER GLUTATHIONE (GSH):-**

Cyclophosphamide administration in the control group lowered the GSH level in the liver tissue (13.630± 2.403 nmoles/mg protein). GALC treatment also showed increased levels of GSH (27.723± 4.481 nmoles/mg protein), but the high dosage regimen, raised the level to normal (value obtained for the normal group was 37.208 ± 5.419 nmoles/mg protein ) which is clearly evident from the GSH reading of GAHC group, 38.546±5.413 nmoles/mg protein (Table 18 & Fig 17).

**Table : 18 -** Effect of *Gmelina arborea* on liver Glutathione (GSH) (nmol/mg protein).

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 37.208 ± 5.419 | 13.630±2.403[a] | 27.723± 4.481[c] | 38.546±5.413[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

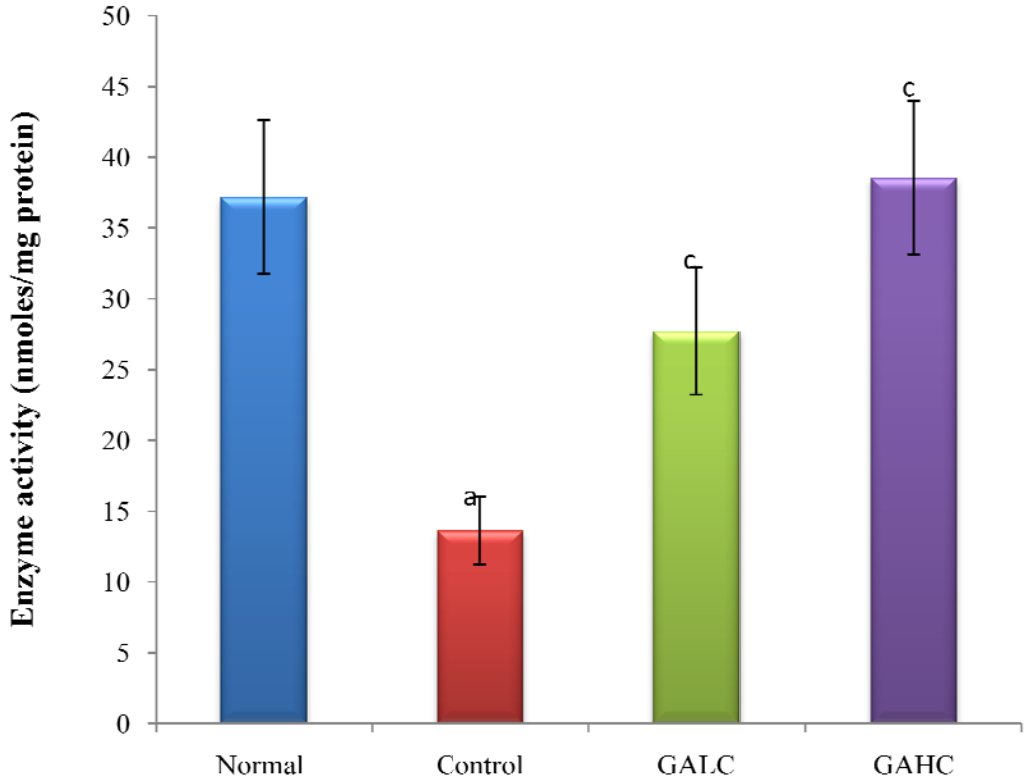



**Fig: 17 -** Effect of *Gmelina arborea* on liver Glutathione (GSH) (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**B.2) ESTIMATION OF KIDNEY GLUTATHIONE (GSH):-**

GSH level was found to be lower in the control group (15.825±3.408 nmol / mg protein). It was replenished to normal range (31.244±6.419 nmol / mg protein) by the high and low doses of GAHC (29.758±4.019 nmol / mg protein) and GALC (19.484±3.682 nmol / mg protein) drug regimen respectively (Table 19 & Fig 18).

**Table : 19 -** Effect of *Gmelina arborea* on kidney Glutathione (GSH) (nmol/mg protein).

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 31.244±6.419 | 15.825±3.408[a] | 19.438±3.682 | 29.758±4.019[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

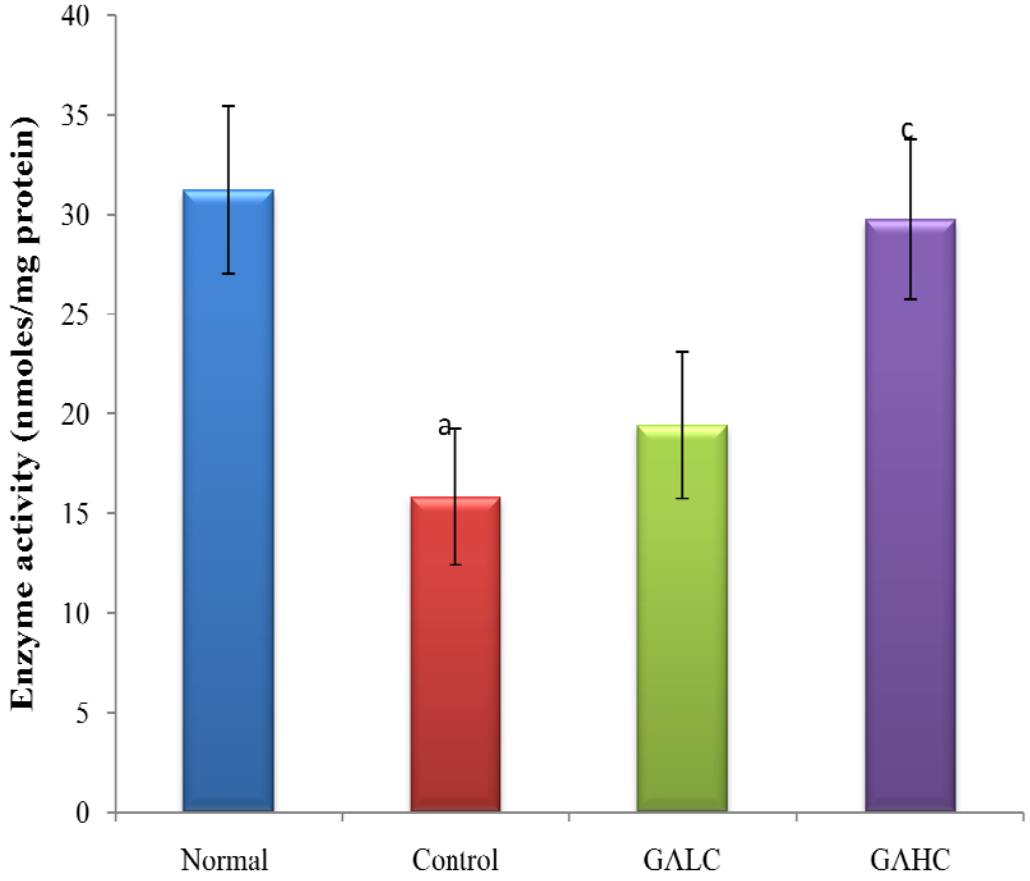



**Fig : 18 -** Effect of *Gmelina arborea* on kidney Glutathione (GSH) :- (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**C.1) ESTIMATION OF LIVER GLUTATHIONE PEROXIDASE (GPX) ACTIVITY:-**

In normal rats liver Glutathione peroxidase level was 10.044±1.094 U/mg, while cyclophosphamide treatment reduced the synthetic activity of the liver leading to a low GPx value of 7.149±1.036 U/mg in the control group. The impact of cyclophosphamide treatment was reduced in the pre-treated GALC and GAHC groups where the Glutathione peroxidase levels were 8.979±0.903 U/mg and 10.026±0.639 U/mg respectively (Table 20 & Fig 19).

**Table : 20** - Effect of *Gmelina arborea* on liver Glutathione peroxidase (Enzyme activity in U/mg protein)

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 10.044±1.094 | 7.149±1.036[a] | 8.979±0.903[d] | 10.026±0.639[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

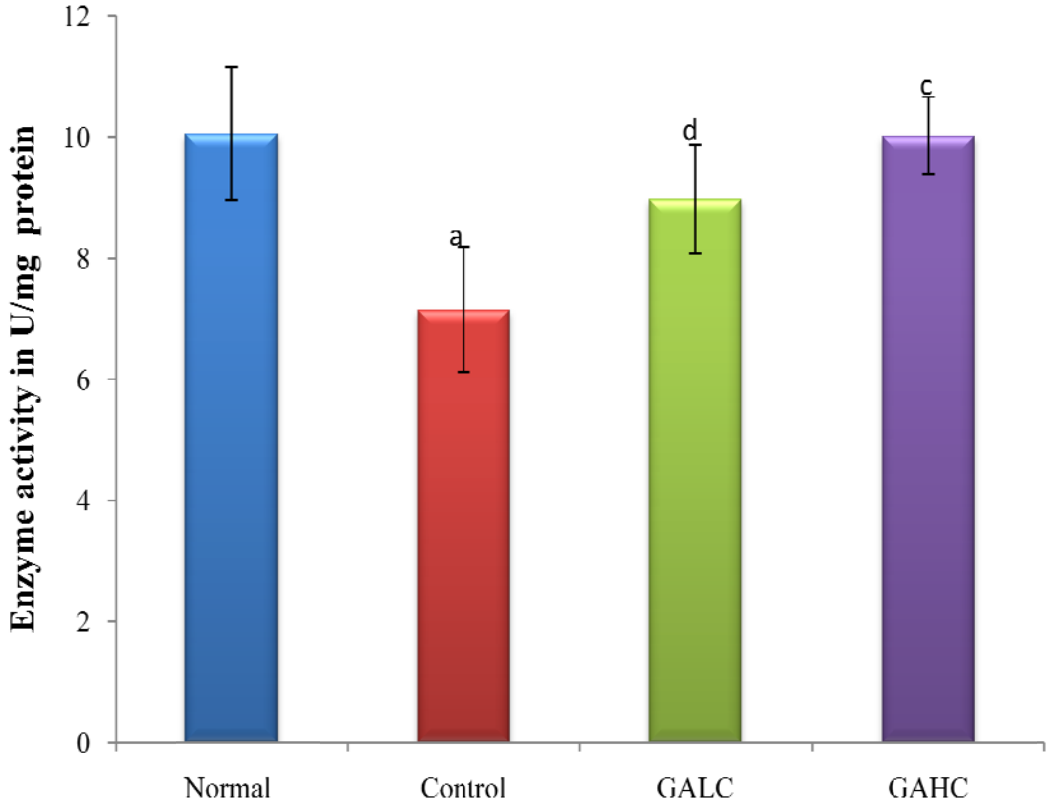



**Fig : 19** - Effect of *Gmelina arborea* on liver Glutathione peroxidase (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal; c:- p<0.01 and d:- p<0.05 compared to control).

## C.2) ESTIMATION OF KIDNEY GLUTATHIONE PEROXIDASE (GPX) ACTIVITY:-

GPX level was found to be lower in the control group (6.754±1.090 U/mg). It was replenished to normal range (10.514±1.269 U/mg) by the GAHC (10.212±0.954 U/mg) and GALC (8.176±0.477 U/mg) drug regimen respectively(Table 21 & fig 20).

**Table : 21 -** Effect of *Gmelina arborea* on kidney Glutathione peroxidase(Enzyme activity in U/mg protein)

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 10.514±1.269 | 6.754±1.090[a] | 8.176±0.477 | 10.212±0.954[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

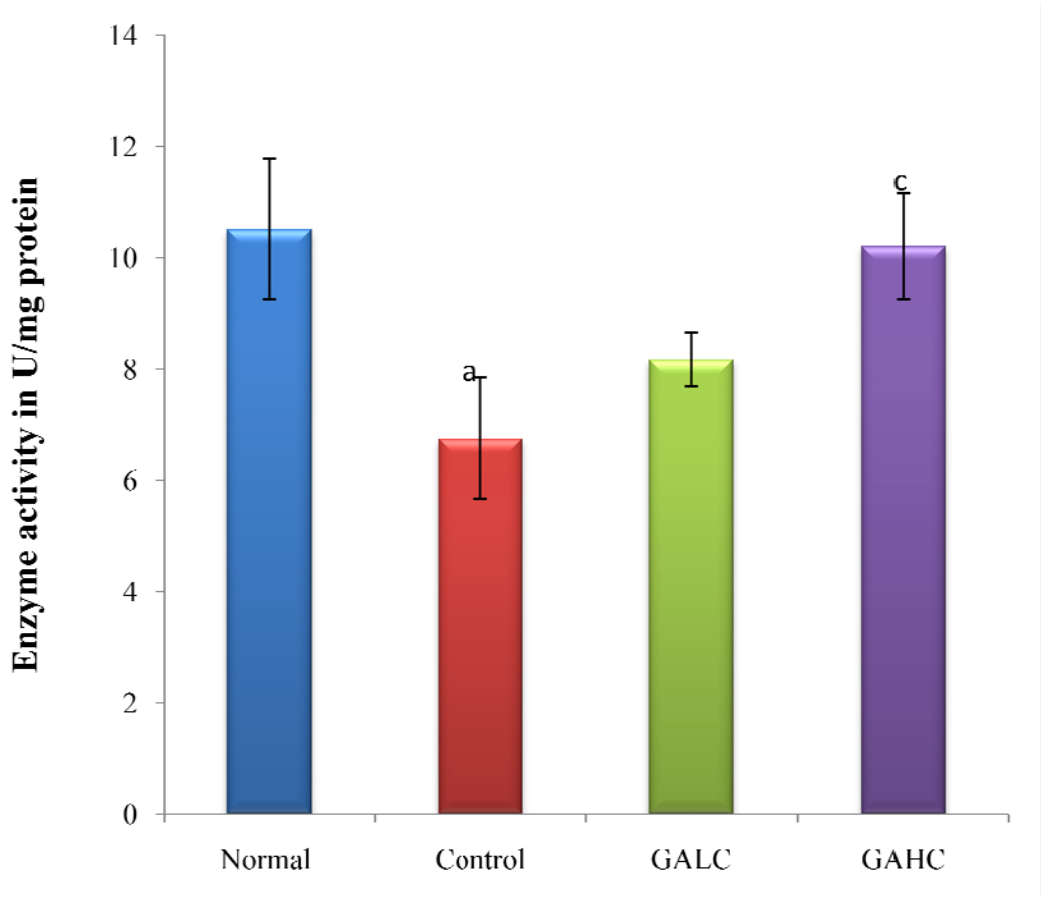



**Fig : 20 -** Effect of *Gmelina arborea* on kidney Glutathione peroxidise (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

## D.1) ESTIMATION OF LIVER TISSUE LIPID PEROXIDATION ACTIVITY:-

The extent of lipid peroxidation in the liver tissue, measured as nmoles of MDA/mg protein, was high in the control group (0.412±0.07 nmoles/mg protien), in comparison to normal (0.257±0.06 nmoles/mg protein). GALC and GAHC pre-treatment evidently reduced the extent of lipid peroxidation in liver tissue. The extent of Lipid Peroxidation in low and high dose groups of GA were 0.352±0.045 nmoles/mg protien and 0.299±0.028 nmoles/mg protien respectively (Table 22 & Fig 21).

**Table :22** - Effect of *Gmelina arborea* on liver tissue lipid peroxidation level (MDA in nmoles/mg protein).

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 0.257±0.06 | 0.412±0.07[a] | 0.352±0.045 | 0.299±0.028[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

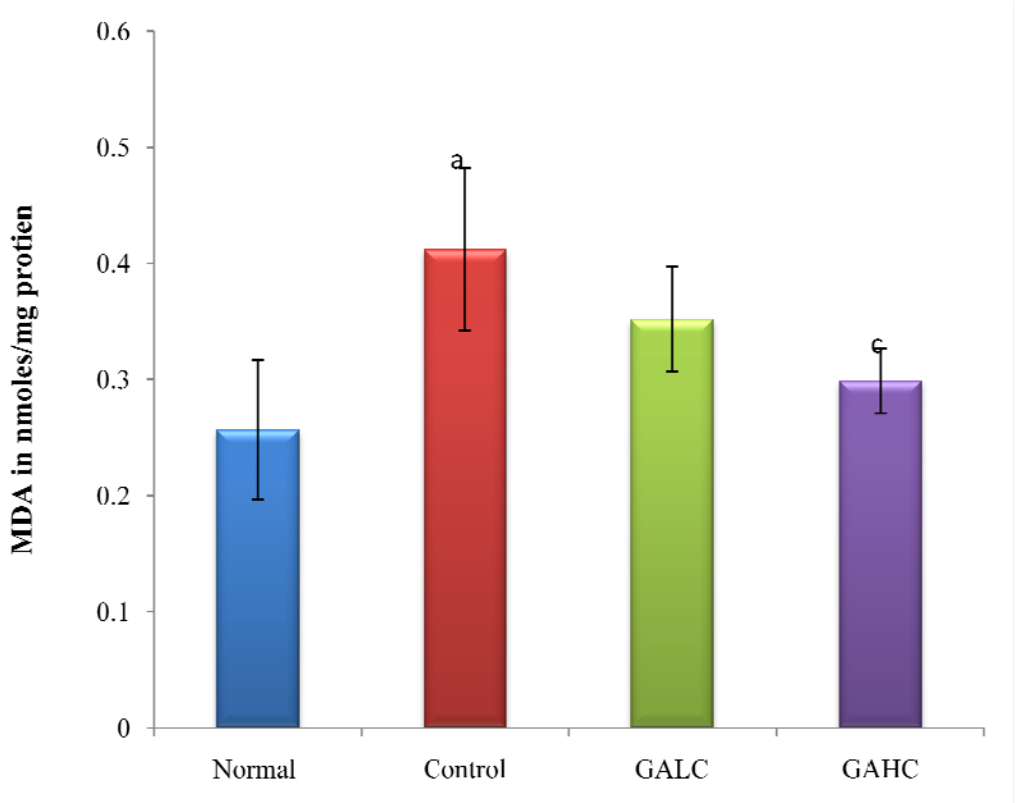



**Fig :21** - Effect of *Gmelina arborea* on liver tissue lipid peroxidation level (Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**D.2) ESTIMATION OF KIDNEY TISSUE LIPID PEROXIDATION :-**

The extent of lipid peroxidation in the kidney tissue, measured as nmoles of MDA/mg protein, was high in the control group (0.419±0.056 MDA in nmoles/mg protien), in comparison to normal (0.200±0.051 MDA in nmoles/mg protein). GALC and GAHC pre-treatment evidently reduced the extent of lipid peroxidation in liver tissue. The extent of Lipid Peroxidation in low and high dose groups of GA were 0.268±0.017 MDA in nmoles/mg protien and 0.249±0.047 MDA in nmoles/mg protien respectively (Table 23 & Fig 22).

**Table :23** - Effect of *Gmelina arborea* on kidney tissue lipid peroxidation level (MDA in nmoles/mg protein).

| NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|
| 0.200±0.051 | 0.419±0.056[a] | 0.268±0.017[c] | 0.249±0.047[c] |

Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control.

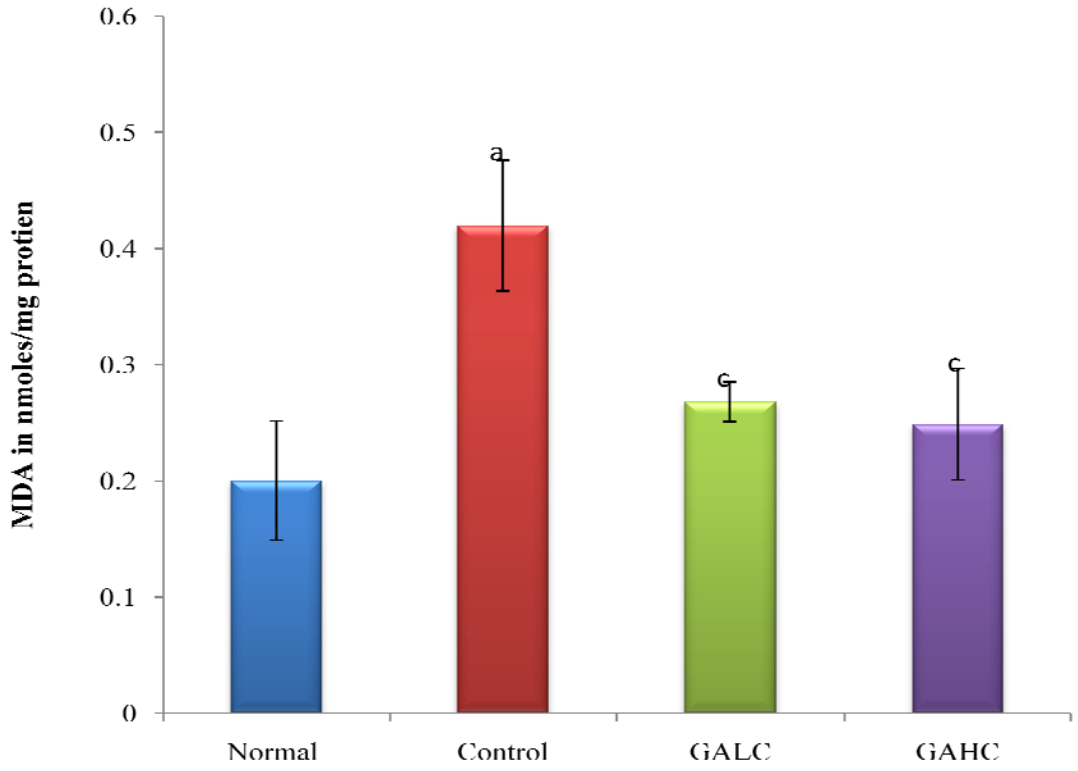



**Fig : 22** - Effect of *Gmelina arborea* on kidney tissue lipid peroxidation level **(**Values are expressed as mean ± SD for 6 animals; a :- p<0.01 and b :- p<0.05 compared to normal ; c:- p<0.01 and d:- p<0.05 compared to control).

**Table :24 -** Effect of *Gmelina arborea* on tissue antioxidant parameters

| TISSUE ANTIOXIDANT PARAMETERS | | NORMAL | CONTROL | GALC | GAHC |
|---|---|---|---|---|---|
| **SOD** (U/mg protein) | **LIVER** | 0.679 ± 0.078 | 0.382± 0.039[a] | 0.509±0.051[c] | 0.58±0.046[c] |
| | **KIDNEY** | 0.805 ± 0.093 | 0.577 ±0.087[a] | 0.68 ±.01 | 0.72 ±0.096[d] |
| **GSH** (nmol/mg protein) | **LIVER** | 37.20 ± 5.419 | 13.630±2.403[a] | 27.723±4.481[c] | 38.546±5.413[c] |
| | **KIDNEY** | 31.244±6.419 | 15.825±3.408[a] | 19.438±3.682 | 29.758±4.019[c] |
| **GPx** (U/mg protein) | **LIVER** | 10.044±1.094 | 7.149±1.036[a] | 8.979±0.903[d] | 10.026±0.639[c] |
| | **KIDNEY** | 10.514±1.269 | 6.754±1.090[a] | 8.176±0.477 | 10.212±0.954[c] |
| **LPO** (MDA in nmol/mg. Protein) | **LIVER** | 0.257±0.06 | 0.412±0.07[a] | 0.352±0.045 | 0.299±0.028[d] |
| | **KIDNEY** | 0.200±0.051 | 0.419±0.056[a] | 0.268±0.017[c] | 0.249±0.047[c] |

Values are expressed as mean ± SD for 6 animals; a :- $p<0.01$ and b :- $p<0.05$ compared to normal ; c:- $p<0.01$ and d:- $p<0.05$ compared to control.

## 9. EFFECT OF GMELINA ARBOREA STEM BARK EXTRACT ON MICE SMALL INTESTINE:

From the histopathological analysis of the intestine of normal mice it was seen that the glands and villi were normal, lined by columnar cells. The muscle layer also appeared normal. But the untreated control group administered with cyclophosphamide alone showed mucosal damage, hyperplasia of the mucosal glands and villi as well as lymphocyte infiltration. The muscle also posses inflammatory changes. Mean while there was minimum damage to the intestinal architecture in GALC treated groups compared to the control. The intestinal section of GAHC showed normal glands, villi and muscles indicating the protective effect of *Gmelina arborea* stem bark extract against cyclophosphamide induced toxicity in mice.

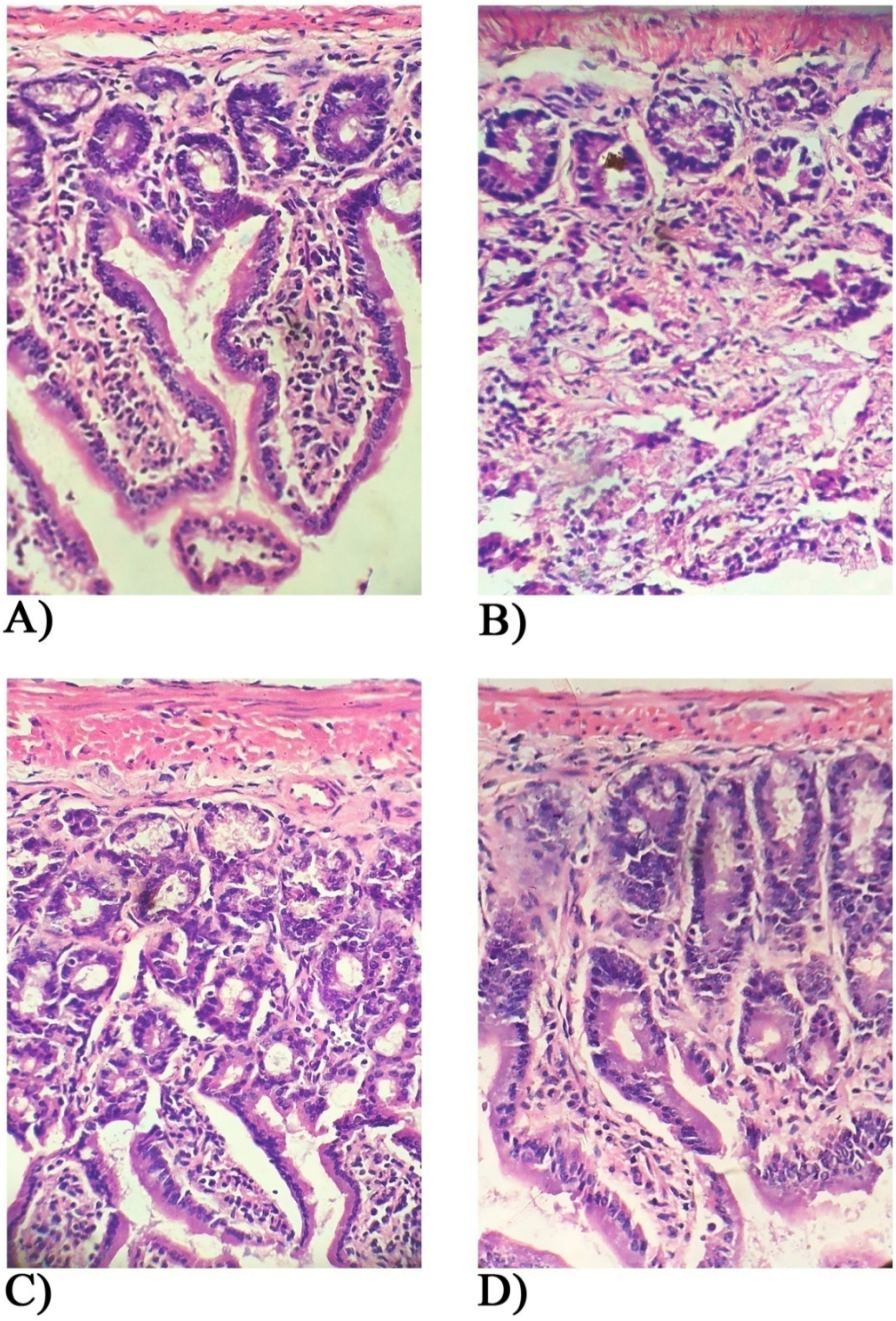



**Fig:23.** Histopathological analysis of mice small intestine**.** (A) Normal (B) Control (C) GALC -250 mg/kg b.wt (D) GAHC - 500 mg/kg b.wt. Hematoxylin and eosin (H&E) staining (40x).

# 8. DISCUSSION

Cancer is one of the most common devastating disease affecting millions of people per year around the world. It has been found that cancer is the second leading cause of death in humans.

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells in our body. The normal control systems in cancer cells that prevent cell overgrowth and the invasion of other tissues are disabled. Treatment for cancer should not begin until the presence of cancer is confirmed by a tissue (i.e., histologic) diagnosis.

Cancer treatment is the series of interventions including psychosocial support, chemotherapy, surgery and radiotherapy that is aimed at curing the disease or prolonging life considerably while improving the patient's quality of life. The course of treatment depends on the type of cancer, location of cancer and its state of advancement.

Chemotherapy drugs are toxic compounds that target cells that are growing rapidly. Chemotherapy can be used to treat many cancers. More than 100 chemotherapy drugs with different mechanism of action are used today, either as single drug or in combination with other drugs or treatments. These drugs vary widely in their chemical composition, formulation, how they are taken, their effectiveness in treating specific forms of cancer and their side effects.

In this present study cyclophosphamide (CTX) has been used as an anticancer agent. Chemoprotective activity of 70 % methanolic extract of *Gmelina arborea* stem bark was studied against the toxicity induced by cyclophosphamide administration. Cyclophosphamide is probably the most commonly used alkylating agent. Cyclophosphamide administration results in the formation of cross-links with DNA by the metabolites produced. Cyclophosphamide undergoes metabolism by microsomal enzymes of the cytochrome $P_{450}$ system to several intermediates with alkylating activity. The principal metabolites identified are phosphoramide mustard and acrolein. Phosphoramide mustard can undergo dephosphoramidation to yield nitrogen mustard, which also has alkylating activity. Metabolites of cyclophosphamide can interact with DNA and a protein, resulting in the formation of adducts (Balu *et al*., 2002). Phosphoramide mustard can generate myelosuppression and haemorrhagic cystitis can be caused by the acrolein. High dose acute acrolein toxicity produces oxidative stress

subsequent to the loss of glutathione (GSH) and alkylation reactions at various nucleophillic sites in cell-particularly in the nucleaus. Low acrolien doses inhibit cell proliferation without affecting viability (Horton N D *et al*., 1997). The *G.arborea* treated groups were found to show almost normal levels of bone marrow cellularity and total WBC count compared to cyclophosphamide treated group thereby illustrating the protective effect of *G. arborea*. The haemoglobin count has no significant difference in all the test groups.

There is pharmacologic evidence that the breakdown of CTX into biologically active alkylating compounds takes place principally in the liver (Brock and Hohorst., 1967). As a result toxic metabolites which are produced in the liver leads to the toxicity of the liver. Hepatic damage is always associated with the cellular necrosis, the increase in tissue lipid peroxidation and the depletion in the tissue glutathione (GSH) levels (Sandy and Ben., 1998). These findings are in agreement with those of the present study, in which there was a significant depletion of GSH and a significant increase in lipid peroxides (MDA) in liver and kidney tissue homogenates of cyclophosphamide treated control group when. While the lipid peroxidation level has been significantly reduced in both liver ($p<0.05$) and kidney ($p<0.01$) tissues of the *Gmelina arborea* high dose treated group.

In order to investigate whether the antioxidant activities of *G. arborea* are mediated by the increase in antioxidant enzyme activity, we measured SOD and GPx activities in different tissues of mice treated with *G. arborea* stem bark extract. In the present study, treatment of mice with *G. arborea* stem bark extract significantly ($p<0.01$) increased the SOD and GPx activities of liver and kidney tissues.

Liver damage was assessed by biochemical studies such as estimation of serum levels of AST, ALT and ALP. Similarly, Merlin and Parthasarathy., 2011 reported the elevation of serum levels of many biochemical markers like aspartate transaminase (AST), alanine transaminase (ALT), serum alkaline phosphatise (ALP), triglycerides, cholesterol and bilirubin. Treatment of mice with cyclophosphamide increased the serum transaminase level whereas animals pretreated with *G. arborea* stem bark extract decreased the elevated levels of transaminases (ALT & AST) in a significant manner.

The excretion of both urea and creatinine depends on the glomerular filtration rate. Hence, when damage occurs to the kidney, its values in serum get increased from the normal. The present study revealed that, there is a significant increase in the serum creatinine and urea level on the animals which is treated with cyclophosphamide alone,

Whereas the *G. arborea* high dose treated group showed a decline in the serum levels of Urea and Creatinine in a significant manner ($p<0.01$).

Changes in the body weight and organ weight of animals were also analysed during the period to find out the toxic effects of cyclophosphamide. Reduction in body weight is observed only in control groups. According to Manson and Kang (1994) and Chahoud *et al*., 1999, the body weight alterations are usually observed indicative of toxicity in mice. No significant reduction in organ weight was noted except spleen weight. In control group animals, reduction in spleen weight was noted when compared to GALC & GAHC treated groups.

In the histopathological studies of small intestine, *G. arborea* treated groups showed no significant structural damage when compared to the cyclophosphamide alone treated control group. In this observation it is assumed that *G. arborea* is highly significant to protect the small intestine form cyclophosphamide toxicity.

These findings clearly demonstrated the chemoprotective activities of *G. arborea* stem bark extract in experimental mice models.

# 9. CONCLUSION

The evidence obtained from the present study indicates that the treatment with extract of *Gmelina arborea* can able to protect the liver and kidney against cyclophosphamide induced toxicity. Restoration of serum parameters, tissue antioxidants and haematological parameters to its normal value and the reduction in the level of tissue lipid peroxidation accounts for the chemoprotective activity of 70% methanolic extract of *Gmelina arborea* stem bark.

There are number of reports evaluating the phytochemical constituents and antioxidant properties of the plant *Gmelina arborea*. However, the components responsible for the chemoprotective activity are currently unclear. Therefore, further investigations are needed to be carried out to isolate, identify the compounds present in the plant extract and to find out possible mechanism(s) of the protective action of *Gmelina arborea.*

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